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## CLINICAL AND EXPERIMENTAL

# MYOCARDIAL INFARCTION: AN ELECTROCARDIOGRAPHIC STUDY*

### A REPORT OF NINE CASES FROM THE MAYO CLINIC, AND A REVIEW OF TWENTY-FOUR PUBLISHED CASES

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THE clinical syndrome accompanying sudden obstruction of the coronary circulation is not always typical, and hence not infrequently leads to diagnostic error. It is important, therefore, that all cases of this type be carefully studied with special reference to the correlation of symptoms, physical findings, and special lines of cardiac investigation, such as electrocardiography and necropsy. The scope of this paper does not permit a detailed discussion of the clinical features of myocardial infarction which have been repeatedly and comprehensively recorded in the literature.[1, 2, 3, 10] But in view of the fact that few representative series of cases with electrocardiographic study have been recorded, we have incorporated our group of nine cases with those already published and base our conclusions on the aggregate group.

#### REPORTS OF CASES (WILLIUS AND BARNES)

CASE 1 (A148902).—A man, aged fifty-seven years, was admitted to the hospital August 15, 1919, suffering with severe epigastric pain. He had had typical attacks of angina pectoris for the last five years, which had increased in frequency and severity during the year preceding this admission. Four years before, the blood pressure was elevated, the systolic pressure being 162 and the diastolic 128. After his noon meal the day before examination, the patient was suddenly taken with severe, localized, persistent pain in the epigastrium. He vomited once, soon after the onset of the pain. His home physician had given six subcutaneous injections of morphine, varying from ¼ to ⅜ grain, without relief.

Examination revealed an extremely ill man, who presented the facies of extreme suffering. The area of cardiac dullness was increased, extending 12 cm. to the left of

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the median sternal line. The heart tones were very distant and heard with great difficulty, owing to noisy breathing. The abdomen was moderately distended and tympanitic, and tender in the epigastrium. The blood pressure was not obtained. The temperature was 102° F., and the leucocyte count was 34,200. The electrocardiogram disclosed T wave negativity in all derivations, with preponderance of the left ventricle (Fig. 1). The patient died one hour after admission to the hospital.

At necropsy the heart was markedly enlarged, the apex consisting largely of the left ventricle. The coronaries were prominent, tortuous and beaded. The aortic valve, especially around the attachments of the leaflets, contained a moderate amount of fatty and fibrous material with some calcareous deposits. The myocardium of the left ventricle was enormously thickened, in some places measuring 2.5 to 3 cm. The muscle was mottled chocolate, yellow and grey. In the upper half of the interventricular septum was a dark reddish area about 2 cm. in diameter, and softer than the rest of the myocardium. A similar area was found on the external surface of the heart on the

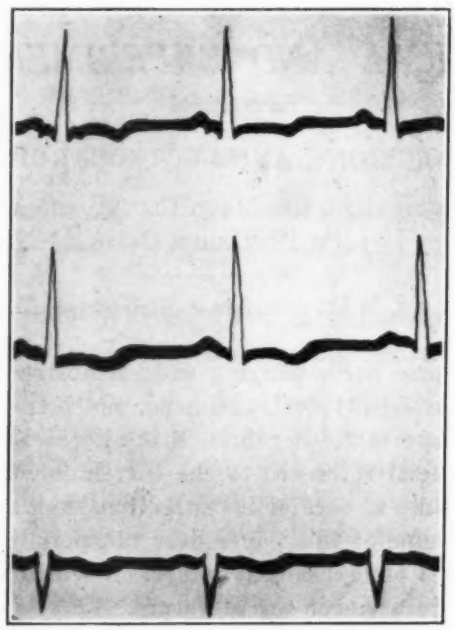

Fig. 1.—Case 1 (A148902). Taken about one-half hour before death. T wave negativity in Derivations I, II and III. Left ventricular preponderance.

posterior surface of the left ventricle, almost at the auriculo-ventricular juncture. A mottled clot was present in the apex of the left ventricle, and firmly attached to the papillary muscles. The coronaries were opened with great difficulty and the walls were found to be greatly thickened and to contain many nodular, yellow and grey plaques. The lumen in some places was practically occluded. Besides the myocardial infarction, there were multiple infarcts in the right kidney, and an embolism in the superior mesenteric artery.

Case 2 (A298068).—A man, aged forty-two years, was admitted to the Clinic November 26, 1919, complaining of attacks of severe retrosternal pain following exertion. He had contracted syphilis in 1896 and received little or no treatment until two years ago. During the last seven years he had had severe attacks of retrosternal pain following exertion, or the ingestion of a rather heavy meal. The attacks had become more frequent and severe during the last three weeks.

Examination revealed a well nourished man, not particularly ill. His heart was moderately enlarged, and to-and-fro murmurs were audible at all areas, maximal at the

aortic area. Capillary and water-hammer pulse, and pistol-shots in the femoral arteries were noted. The liver edge was palpable 2 cm. below the right costal margin. The systolic blood pressure varied from 128 to 136, and the diastolic was 60. The urinalyses were negative. The hemoglobin was 74 per cent, the erythrocytes numbered 4,600,000, and the leucocytes 9,200. The blood Wassermann reaction was negative. Roentgenograms of the chest revealed moderate enlargement of the heart, particularly to the left. The first electrocardiogram taken three days after admission to the hospital showed a rate of 130, sinus tachycardia, negative T wave in Derivation I, and preponderance of the left ventricle. The T wave in Derivations II and III was markedly exaggerated (Fig. 2). A diagnosis was made of syphilitic aortitis with aortic regurgitation, myocardial degeneration with dilatation and hypertrophy, and angina pectoris.

The patient became progressively worse, and died of a progressive cardiac failure nine days after admission to the hospital. There was no fever, but the leucocyte count

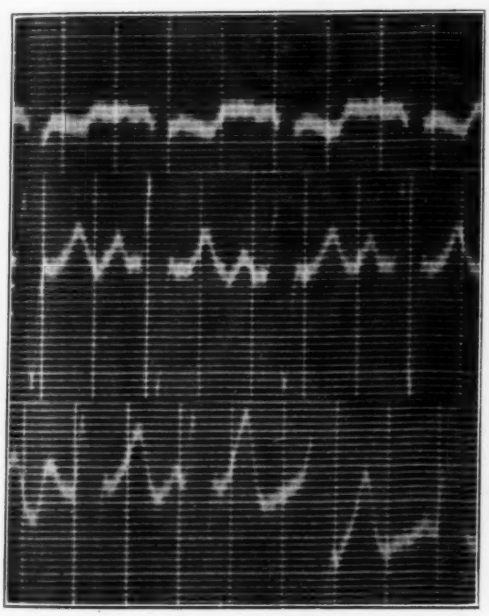

Fig. 2.—Case 2 (A298068). Taken three days after admission. T wave negativity in Derivation I. Left ventricular preponderance.

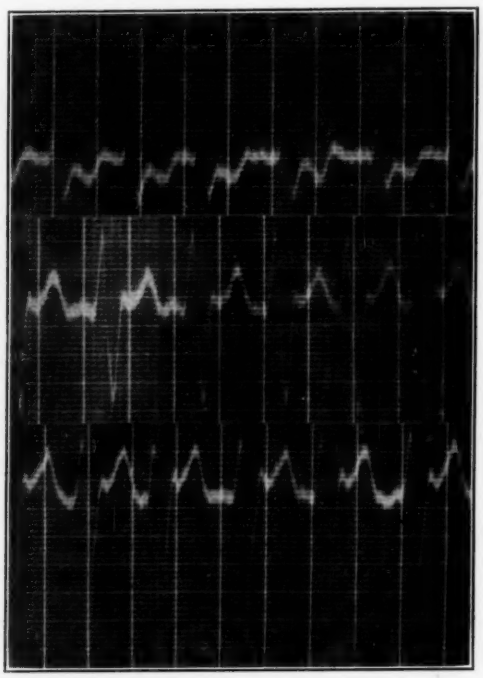

Fig. 3.—Case 2 (A298068). Taken four days after tracing in Fig. 2, and on the day preceding death. Rate 166. Nodal tachycardia. T wave negativity in Derivation I. Left ventricular preponderance.

the day before death was 16,600. The electrocardiogram taken on the same day showed a rate of 166, nodal tachycardia, occasional ventricular premature contractions, T wave negativity in Derivation I, and preponderance of the left ventricle (Fig. 3).

At necropsy advanced syphilitic aortitis was present. The anterior aortic cusp was scarred and somewhat adherent. The aorta from without was studded with oval projections, which were evidently early aneurysmal pocketings. In the apex of the left ventricle in the visceral pericardium was a hemorrhagic infarct about 2 cm. in diameter, which was much softer than the contiguous structures, and extended to the depth of 2 cm. The coronary arteries were patent and showed no sclerosis.

Case 3 (A399583).—A man, aged sixty-five years, came to the Mayo Clinic July 28, 1922, complaining of attacks of retrosternal pain with radiation into the left arm on exertion. His only previous illnesses had been typhoid fever at the age of twenty-six,

and pneumonia six years before his examination. The attacks of retrosternal pain were becoming more frequent and severe, and at times he had severe attacks of dyspnea in which he feared he would die.

Examination revealed a well-developed man who was able to be up and about with relative comfort. The cardiac dullness was increased, extending 3 cm. to the right and 15 cm. to the left of the median sternal line. The rhythm was regular, but to-and-fro murmurs were audible at all areas; both were maximal at the aortic area. A capillary and water-hammer pulse was present, and pistol-shots were audible in the femoral arteries. The lungs were emphysematous. The prostate was slightly enlarged, firm and smooth. The systolic blood pressure was 192; it was impossible to determine the diastolic reading accurately. A detailed neurologic examination disclosed the findings of syphilis of the central nervous system.

The blood Wassermann reaction was strongly positive. The urinalyses revealed a small amount of albumin, an occasional hyaline and granular cast and a few erythrocytes and leucocytes. The hemoglobin was 85 per cent, the erythrocytes numbered 4,500,000

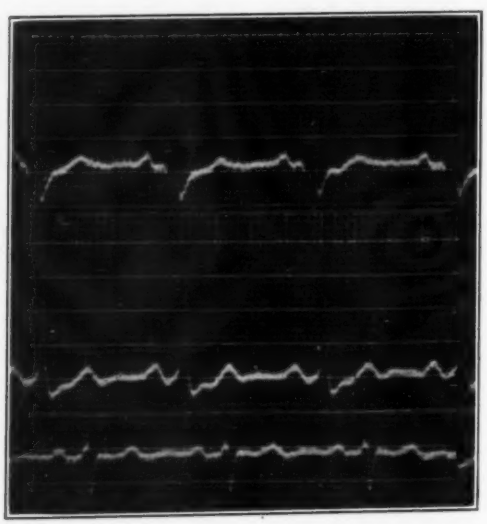

Fig. 4.—Case 3 (A399583). Taken three days after admission and twenty-three days before death. Sinus rhythm. Left ventricular preponderance.

and the leucocytes 6,200. The combined phenolsulphonephthalein test gave an excretion of 80 per cent of the dye in two hours. There were 34 mg. of urea and 15.8 mg. of urea nitrogen in each cubic centimeter of blood. Examination of the eyes revealed the Argyll-Robertson type of pupil and the fundi showed mild arteriovenous compression. Roentgenograms of the chest showed only the cardiac enlargement. An electrocardiogram taken three days after admission to the hospital, and twenty-three days before death, revealed only preponderance of the left ventricle (Fig. 4). A diagnosis of syphilitic aortitis and aortic regurgitation was made. The patient grew progressively worse and died of progressive cardiac failure August 23, 1922.

Necropsy revealed that the enlarged heart weighed 500 gm. The myocardium was flabby and on cut section had a mottled appearance. Over the right side of the anterior surface of the epicardium was a white opaque patch measuring 3 cm. by 2 cm. (soldier's patch). There was a diffuse thickening of the endocardium of the left ventricle, and over this were scattered small areas of erosion and ulceration and one large area measuring 3 by 1 cm. to which was attached a thrombus. This area had the appearance of an infarction. The aortic valve showed diffuse thickening and shortening of its cusps. The coronaries were markedly thickened with distinct encroachment of the lumen. The

aorta showed advanced arteriosclerotic changes, especially at the root with atheromatous ulcers, and mural thrombosis.

CASE 4 (A401659).—A man, aged seventy years, came to the Mayo Clinic August 16, 1922. His previous illnesses had been unimportant, other than malaria when a young man and influenza in 1918. He had been pronounced perfectly well three months before examination, and readily passed two life insurance examinations. Two weeks before, while undressing, he had a sudden, severe, agonizing pain behind the sternum and upper abdomen; his suffering was so extreme that he thought he was going to die. This pain lasted three days.

The patient had another attack of pain, identical to the first, the day after his arrival at the Clinic. He was admitted to the hospital at once. His temperature was

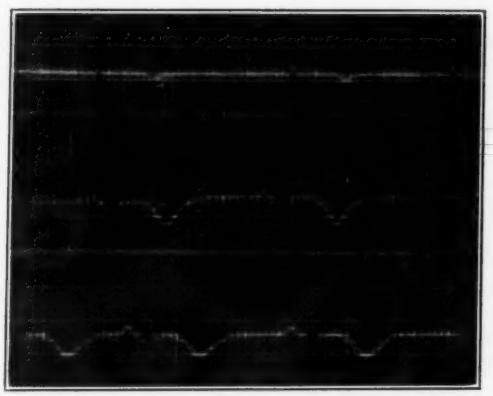

Fig. 5.—Case 4 (A401659). Taken during attack. T wave negativity in Derivations I, II and III.

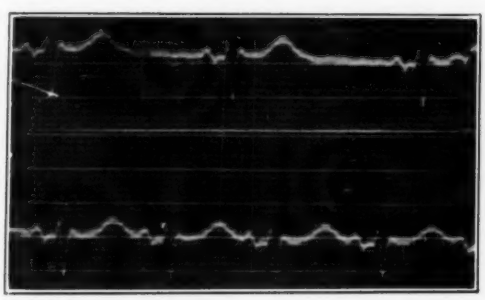

Fig. 6.—Case 4 (A401659). Taken same day as tracing in Fig. 5. Sino-auricular block. Both records in Derivation III.

101° F. His heart was greatly enlarged on the left, but the rhythm was regular and there were no murmurs. Morphine was given with only slight relief. An electrocardiogram taken at this time showed the T wave to be negative in all derivations, and periods of sino-auricular block and preponderance of the left ventricle (Figs. 5 and 6). The heart was definitely enlarged to the left, and an arrhythmia was present. The next day a distinct pericardial rub was audible over the lower sternum. The temperature receded to 99.8° on the second and third days, and then remained normal. The urinalysis was negative and the combined phenolsulphonephthalein test showed an excretion of 50 per cent of the dye in two hours. The blood Wassermann reaction was negative. The roentgenogram confirmed the cardiac enlargement. The blood count was normal. The systolic blood pressure was 108, and the diastolic 78. A diagnosis of acute coronary occlusion was made.

The patient recovered uneventfully and returned home at the end of two weeks. He returned for examination about seven months later (March 13, 1923) complaining of considerable shortness of breath on effort, and attacks of paroxysmal dyspnea at night. There had been no recurrence of anginal attacks.

Examination revealed the heart to be smaller than on the previous examination, the cardiac dullness extending 3.5 cm. to the right and 11 cm. to the left of the median sternal line. There was no arrhythmia and no murmurs. The systolic blood pressure was 110 and the diastolic 70. The electrocardiogram at this time showed the T wave to be negative in Derivation I, and left ventricular prèponderance (Fig. 7).

CASE 5 (A42825).—A man, aged fifty-three years, came to the Mayo Clinic August 30, 1922. He had felt well up to July 1 except for mild indigestion to which he had been subject for ten years. On this day a sudden severe pain developed in the epigastrium, extending over the whole abdomen; the exact duration was not noted. Such attacks recurred at irregular intervals; the dyspnea developed, and was becoming progressively worse at the time of his admission.

On examination the patient appeared to be extremely ill. He was dyspneic, moderately cyanosed, and the dependent portions of the body were markedly edematous. The heart was enlarged, the dullness extending 4.5 cm. to the right, and 14.5 cm. to the

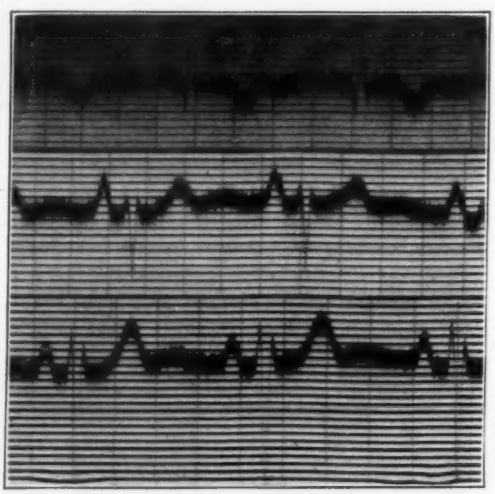

Fig. 7.—Case 4 (A401659). Taken seven months after tracing in Fig. 6. T wave negativity in Derivation I. Left ventricular preponderance.

left of the median-sternal line. There was total arrhythmia. The systolic blood pressure was 120 and the diastolic 100. A twenty-four hour specimen of urine contained 750 c.c., specific gravity 1.018, a faint trace of albumin, no sugar and an occasional leucocyte. The combined phenolsulphonephthalein test showed an excretion of only 15 per cent of the dye in two hours. The urea varied from 82 to 46 mg. in each 100 c.c. of blood. The roentgenogram of the chest confirmed the finding of cardiac enlargement, and showed a thickening of the pleura at both bases. The initial electrocardiogram taken on the third day after the patient's admission to the hospital (September 2, 1922) disclosed auricular flutter, the ventricular rate varying from 83 to 169, and the auricular rate from 327 to 338 (Fig. 8). Three days later the electrocardiogram revealed nodal tachycardia (Fig. 9). Two days later premature contractions were present and there was a tendency for fusion of the R and T waves in Derivations I and II (Fig. 10). The next day (the day of the patient's death), a delay in A-V conduction was noted, the P-R interval being 0.24 second (Fig. 11). This was probably a digitalis effect, as the patient had received 23 c.c. of the tincture in five days.

The patient was placed on the usual cardiac regimen, including the administration of the tincture of digitalis, but became progressively worse and died on the ninth day after admission to the hospital (September 7, 1922).

At necropsy the heart was markedly enlarged, weighing 925 gm. A large globular mass was visible at the apex, which measured 12 cm. in its greatest diameter. The pericardium was adherent to the anterior surface of this mass by dense fibrous adhesions,

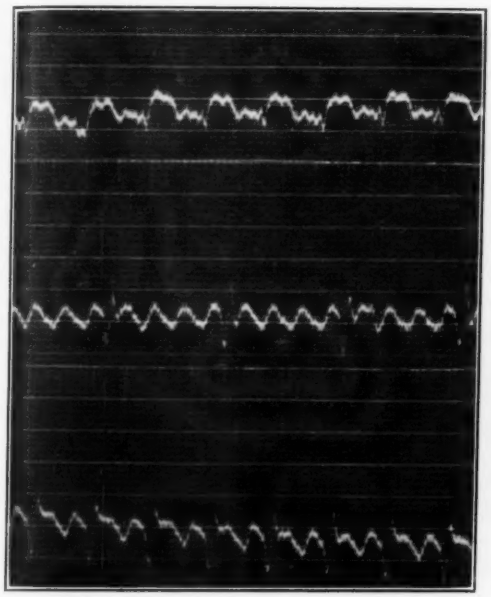

Fig. 8.—Case 5 (A42825). Taken three days after admission. Auricular flutter, ventricular rate 83 to 169, auricular rate 327 to 338 (2:1 to 4:1 block).

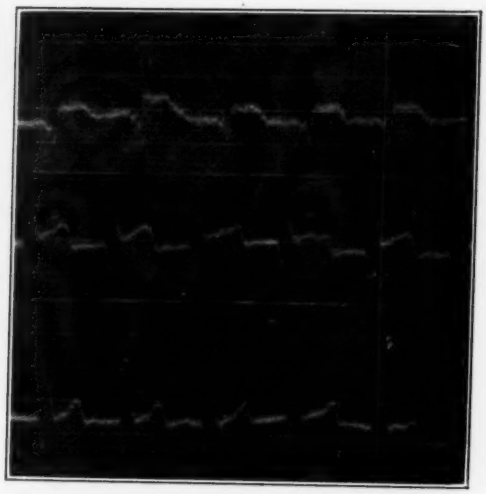

Fig. 9.—Case 5 (A42825). Taken three days after tracing in Fig. 8. Nodal tachycardia.

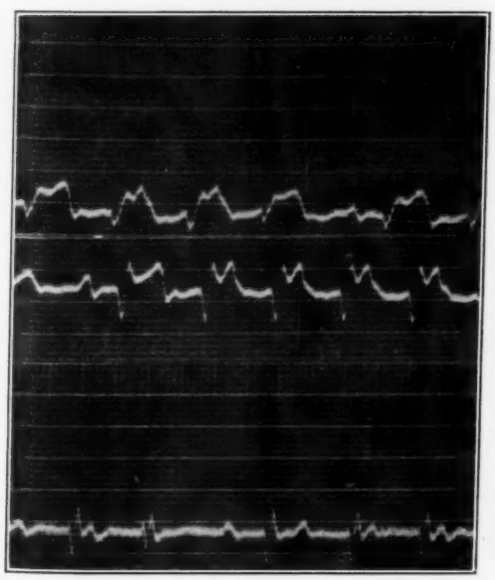

Fig. 10.—Case 5 (A42825). Taken two days after tracing in Fig. 9. Premature contractions. Tendency for fusion of R and T waves in Derivations I and II.

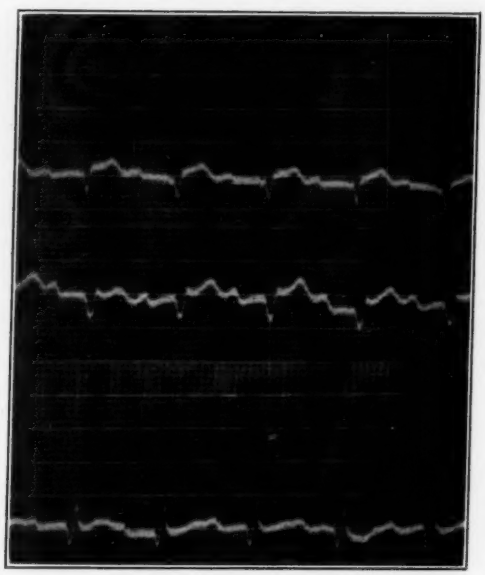

Fig. 11.—Case 5 (A42825). Taken day after tracing in Fig. 10. Delayed A-V conduction (P-R, 0.24 second).

and the wall of the mass consisted of a thin membrane. The anterior portion of the apex and the apex itself of the left ventricle were involved in this outpouching, and out of this pouch a round, flattened thrombus about 10 by 10 cm. came away. The margin of the pouch was sharply demarcated to the left ventricle, but a portion of the interventricular septum adjacent to it was whitish grey and firm. The descending branch of the left coronary artery was completely occluded by an old thrombus, just at the upper margin of the outpouching described.

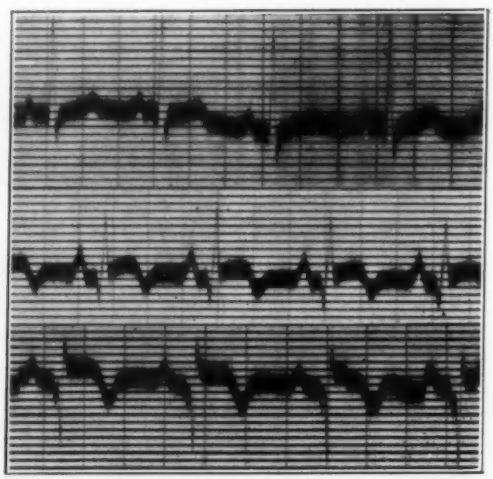

Fig. 12.—Case 6 (A425425). Taken two days after admission and before attack. T wave negativity in Derivations II and III.

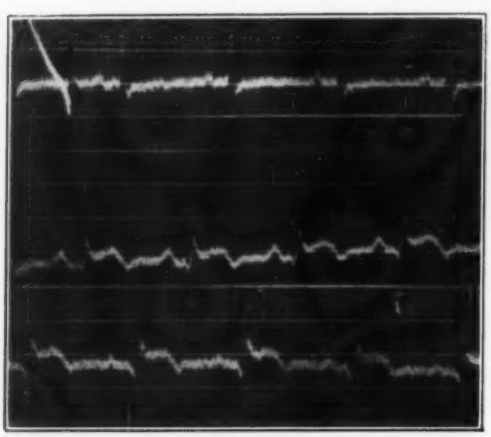

Fig. 13.—Case 6 (A425425). Taken during attack and four days after tracing in Fig. 12. Tendency for fusion of R and T waves in Derivations II and III. T wave negativity in Derivations II and III.

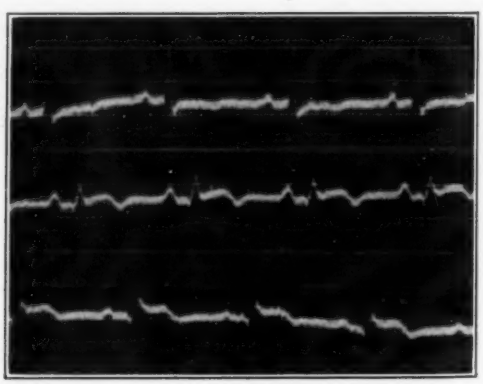

Fig. 14.—Case 6 (A425425). Taken day after tracing in Fig. 13. T wave negativity in Derivations I and II, and diphasic in Derivation III. Fusion of R and T waves has disappeared.

CASE 6 (A425425).—A man, aged forty-three years, was admitted to the hospital May 10, 1923, suffering from severe attacks of retrosternal pain. He said that his general health had been excellent until the onset of the present trouble in March, 1922, when suddenly, while exerting himself, he had a severe retrosternal pain which radiated over the chest, through to the back, and down both arms. This was relieved in a few moments by complete rest. Many similar attacks occurred afterward, following effort, and increased in severity.

Examination revealed the heart to be slightly enlarged, the cardiac dullness extending 3 cm. to the right and 11 cm. to the left of the median sternal line. There was no arrhythmia, and no murmurs. The sytolic blood pressure was 116, and the diastolic 84. There was a moderate degree of peripheral arteriosclerosis. The urinalysis revealed only a faint trace of albumin. The blood Wassermann reaction was negative, and there were 32 mg. of urea in each 100 c.c. of blood; the x-ray confirmed the slight cardiac enlargement.

The electrocardiogram taken two days after admission revealed T wave negativity in Derivations II and III (Fig. 12). A very severe anginal attack occurred which lasted for thirty hours, and during this time the leucocyte count varied from 20,800 to 15,200, and the temperature from normal to 101.2°. The electrocardiogram taken during the attack showed the T wave to be negative in Derivations II and III, but in these derivations there was a tendency for the fusion of the R and the T waves (Fig. 13), and a diagnosis of coronary thrombosis was made. After this severe attack, a distinct to-and-fro pericardial rub was audible to the left of the lower sternum. The electro-

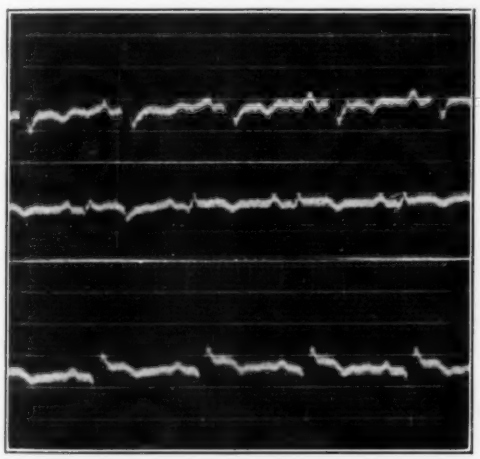

Fig. 15.—Case 6 (A425425). Taken day after tracing in Fig. 14. T wave negativity in Derivations I, II and III.

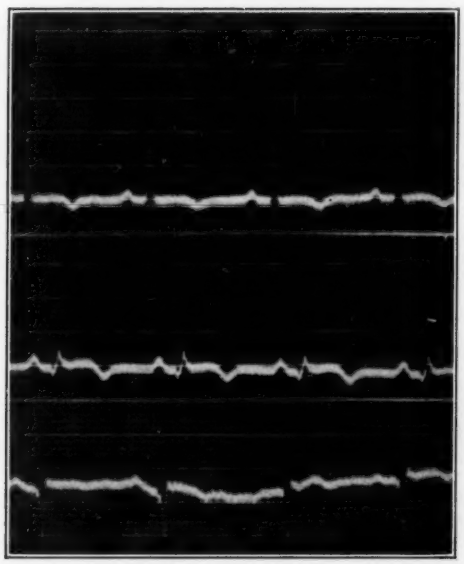

Fig. 16.—Case 6 (A425425). Taken day after tracing in Fig. 15. T wave negativity in Derivations I, II and III.

cardiogram taken the next day showed the T wave to be negative in Derivations I and II, and diphasic in Derivation III; the fusion of the R and the T waves had disappeared (Fig. 14). On the second day, the T wave was negative in all derivations, (Fig. 15) and on the third, the same electrocardiographic findings were present (Fig. 16).

The patient recovered and was alive about six months ago, when he was last heard from.

CASE 7 (A448624).—A man, aged fifty-seven years, was admitted to the hospital November 26, 1923. He had been well except for an attack of mild influenza during the epidemic of 1917. For a year and a half before coming to the Clinic he had complained of shortness of breath on effort. This was moderate until two months prior to his admission, when under relatively little effort he became very dyspneic, particularly when he exerted himself soon after eating. In the early part of November, while driving his automobile, he suddenly developed a severe oppression in his chest, associated with marked difficulty in breathing, and on returning home he went to bed, and remained there for three days. On the day of his arrival at the Clinic, he had an attack

of severe retrosternal pain, associated with marked shortness of breath, which lasted for ten hours.

Examination revealed an enlarged heart, the cardiac dullness extending 3.5 cm. to the right and 11 cm. to the left of the median sternal line, rapid and distant heart tones, and a moderate degree of peripheral arteriosclerosis. The systolic blood pressure was 130, and the diastolic 90. The urinalysis was negative. The hemoglobin was 75

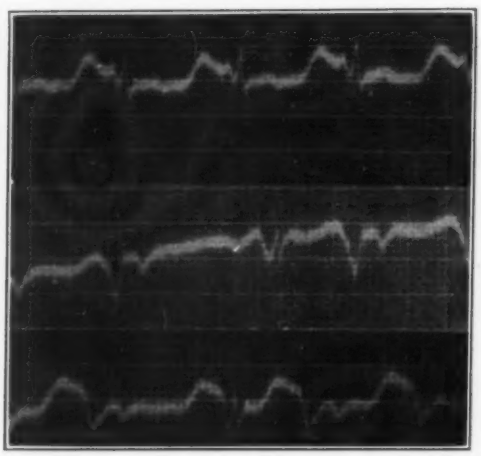

Fig. 17.—Case 7 (A448624). Taken during attack. Ventricular premature contractions. Notched QRS complexes in Derivations I and II, 0.12 second. T wave negativity in Derivations II and III.

Fig. 18.—Case 7 (A448624). Taken day after tracing in Fig. 17. Notched QRS complexes in Derivations I and II, 0.12 second. T wave negativity in Derivations I, II and III.

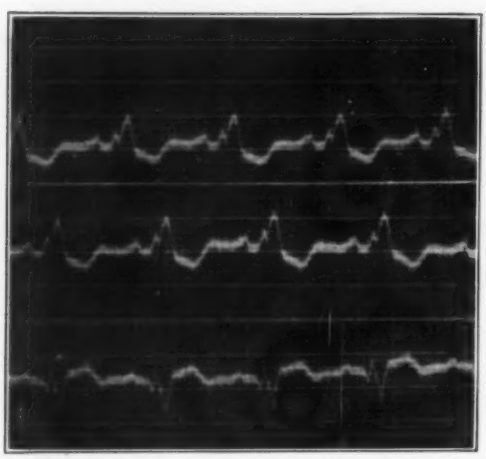

Fig. 19.—Case 7 (A448624). Taken two days after tracing in Fig. 18 and two days before death. Notched QRS complexes in all derivations, 0.12 second. T wave negativity in Derivations I and II.

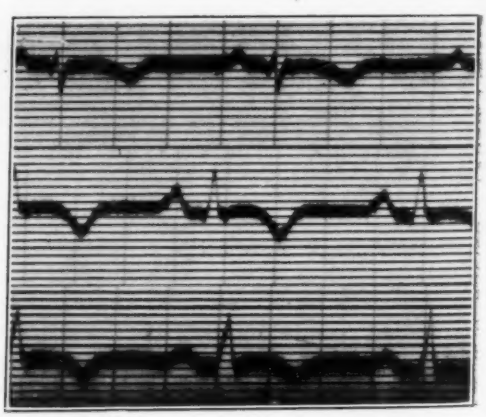

Fig. 20.—Case 8 (A130266). Taken forty-six days after attack. T wave negativity in Derivations I, II and III.

per cent; the erythrocytes numbered 4,840,000, and the leucocytes 7,500. An electrocardiogram taken during this attack revealed ventricular premature contractions, notching of the QRS complexes in Derivations I and II, the base width being 0.12 second (Fig. 17). There was T wave negativity in Derivations II and III. A diagnosis of acute coronary occlusion was made. The patient felt fairly comfortable the next day, and the electrocardiogram was similar to the one taken during the attack, except that the T wave

was negative in all derivations (Fig. 18). The electrocardiogram taken two days later showed a notching of the QRS complexes in all derivations, the base width being 0.12 second with T wave negative in Derivations I and II (Fig. 19). Two days later the patient suddenly developed another severe anginal attack and died in a few moments.

At necropsy the heart was found to be markedly dilated, and weighed 520 gm. The myocardium was firm and light red, and in the anterior surface of the left ventricle a sharply defined area of bright red, 2.5 cm. in diameter was found, which was distinctly

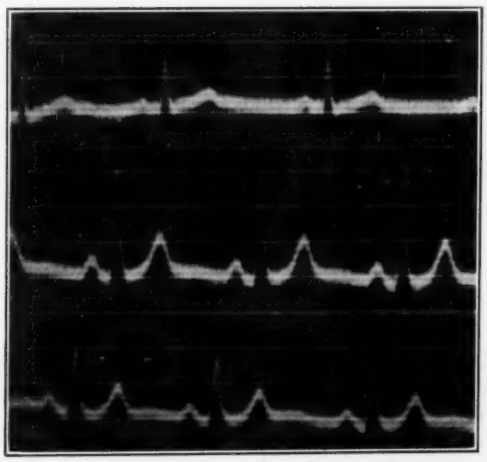

Fig. 21.—Case 9 (A461768). Taken during attack. Sinus arrhythmia.

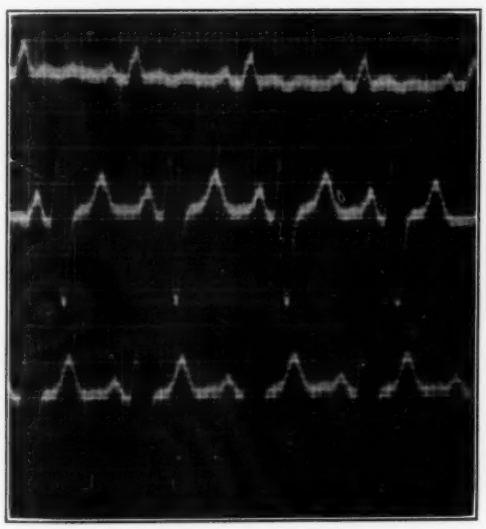

Fig. 22.—Case 9 (A461768). Taken day after tracing in Fig. 21. Nodal premature contractions.

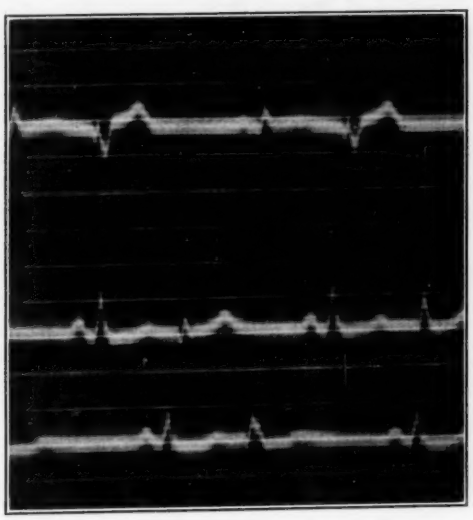

Fig. 23.—Case 9 (A461768). Taken day after tracing in Fig. 22. T wave negativity in Derivation I. Right bundle branch block.

a recent infarction. On the posterior portion of the left ventricular septum was found a scarred and thinned-out area about 1.5 cm. in diameter, the evidence of a previous infarction. There was marked sclerosis of the coronary arteries and a moderate degree of sclerosis at the base of the aorta.

Case 8 (A130266).—A woman, aged sixty years, came to the Mayo Clinic December 6, 1923. The past history was unimportant; however, she had had a cholecystectomy in

September, 1917, for acute empyema of the gall bladder with cholelithiasis, from which she made an excellent recovery. Forty-six days prior to her examination at the Clinic, she had a sudden severe attack of retrosternal pain radiating into both arms, associated with nausea and vomiting, and intense pain for about eighteen hours. The next day her local physician discovered the presence of a pericardial rub, and the condition was interpreted as an acute pericarditis. She was kept in bed for a complete rest for three weeks. The week prior to her visit to Rochester she had two rather severe attacks of shorter duration, but at the time she was under our observation she was entirely free from pain, although under moderate effort, dyspnea was marked.

The heart was slightly enlarged, the cardiac dullness extending 4.5 cm. to the right and 12 cm. to the left of the median sternal line. There were no murmurs, but the tones were rather distant. The urinalysis was negative; the hemoglobin was 74 per cent, erythrocytes numbered 4,730,000, and the leucocytes 9,000. Roentgenograms of the chest confirmed the finding of cardiac enlargement. The electrocardiogram revealed T wave negativity in all derivations (Fig. 20). A diagnosis of previous myocardial infarction was made.

CASE 9 (A461768).—A man, aged forty-seven years, came to the Mayo Clinic with his wife, who was the patient. One morning while here, following an unusually heavy

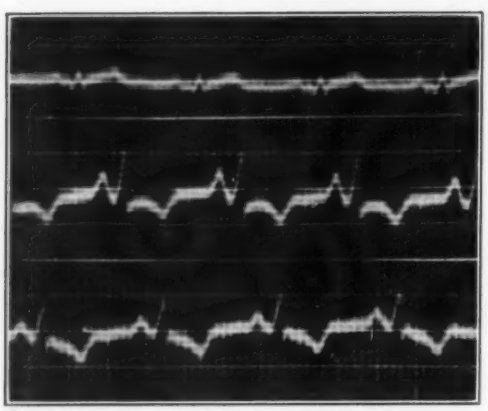

Fig. 24.—Case 9 (A461768). Taken day after tracing in Fig. 23. T wave negativity in Derivations II and III.

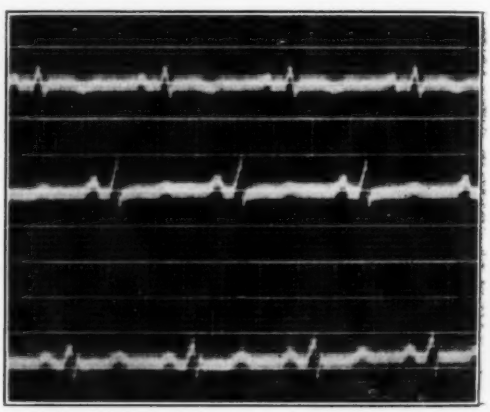

Fig. 25.—Case 9 (A461768). Taken four days after tracing in Fig. 24. T wave negativity in Derivation I.

breakfast, he suddenly developed a severe low substernal and high epigastric pain, radiating into the left arm. He was given 1/175 gr. of nitroglycerin without relief and was immediately sent to the hospital.

The general examination was essentially negative. The heart was not enlarged; there were no murmurs, but a slight respiratory arrhythmia. An electrocardiogram taken about fifteen minutes after his admission to the hospital revealed a sinus arrhythmia (Fig. 21). The patient was given repeated injections of morphine hypodermically without relief. The attack subsided after seven hours. A diagnosis of coronary thrombosis was made.

The next day the electrocardiogram revealed occasional nodal premature contractions, but no significant abnormalities (Fig. 22). The heart was still objectively negative, but the leucocyte count was 12,600 and the temperature was 100.6°. The electrocardiogram, taken the next day, showed T wave negativity in Derivation I, with evidence of right bundle branch block, and was in all characteristics different from those on the preceding days (Fig. 23). Daily electrocardiograms were taken, but only the definite transitions will be recorded. On the next day the electrocardiogram revealed T wave negativity in Derivations II and III, and the curves were again markedly

different from those on the preceding day (Fig. 24). During this period the patient remained comfortable; there was no change in the objective heart findings; and it was not possible to elicit a pericardial rub. Four days later the electrocardiogram had again changed, the T wave being negative in Derivation I (Fig. 25). Nine days later the T wave was still negative in Derivation I, but showed greater amplitude (Fig. 26), and the electrocardiogram taken twelve days later was similar, but also showed ventricular premature contractions (Fig. 27). The last tracing, taken four days later, and the day of the patient's dismissal, was the same (Fig. 28). The heart at this time was slightly enlarged, the tones rather distant and without the clear-cut differentiation of the normal heart tones. The patient was up and about for part of the day, but responded poorly to slight cardiac overload.

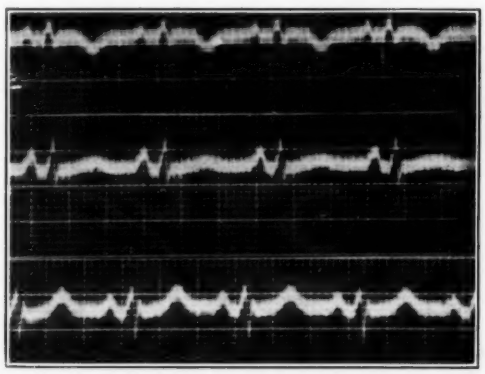

Fig. 26.—Case 9 (A461768). Taken nine days after tracing in Fig. 25. T wave negativity in Derivation I.

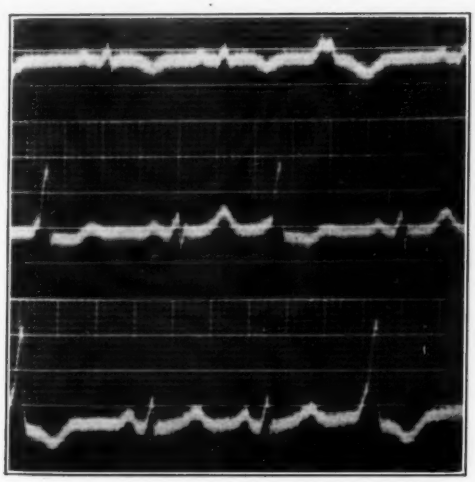

Fig. 27.—Case 9 (A461768). Taken twelve days after tracing in Fig. 26. T wave negativity in Derivation I. Ventricular premature contractions.

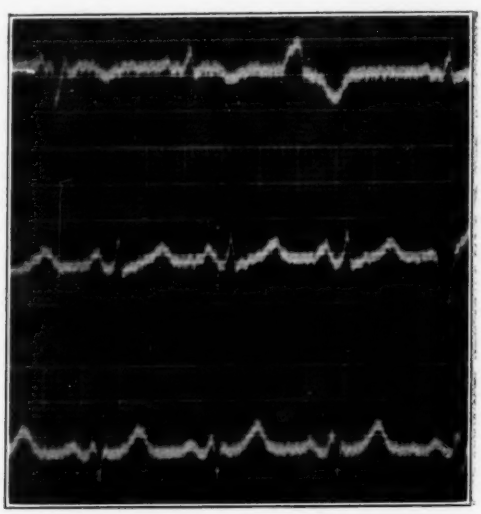

Fig. 28.—Case 9 (A461768). Taken four days after tracing in Fig. 27. T wave negativity in Derivation I. Ventricular premature contractions.

Five of the nine patients in this series died; postmortem examination was made in all.

### DISCUSSION OF ELECTROCARDIOGRAMS

The most constant and striking changes occurring in the electrocardiograms were those affecting the T wave; such abnormalities occurred in eight

TABLE I

MYOCARDIAL INFARCTION (SERIES OF WILLIUS AND BARNES)

| CASE | SEX AND AGE | TRACING | ELECTROCARDIOGRAM | REMARKS |
|---|---|---|---|---|
| 1 (A148902) | M 57 | 1 | Taken about one-half hour before death, T wave negativity in Derivations I, II and III. Left ventricular preponderance | Necropsy. Infarct in the upper half of the interventricular septum |
| 2 (A298068) | M 42 | 1 | Taken three days after admission and about three and one-half weeks after history of occlusion. T wave negativity in Derivation I. Left ventricular preponderance | Necropsy. Hemorrhagic infarct of the apex of left ventricle |
| | | 2 | Taken four days after first tracing and on the day preceding death. Nodal tachycardia, T wave negativity in Derivation I, left ventricular preponderance | |
| 3 (A399583) | M 65 | 1 | Taken three days after admission and twenty-three days before death. Sinus rhythm. Left ventricular preponderance | Necropsy. Infarct of left ventricle |
| 4 (A401659) | M 70 | 1 | Taken during attack. T wave negativity in Derivations I, II and III | Patient recovered |
| | | 2 | Taken same day as first tracing. Sinoauricular block (only Derivation III taken) | |
| | | 3 | Taken seven months after first tracing. T wave negativity in Derivation I, left ventricular preponderance | |
| 5 (A42825) | M 53 | 1 | Taken three days after admission and sixty days after attack. Auricular flutter, ventricular rate 83 to 169, auricular rate 327 to 338 (2:1 to 4:1 block) | Necropsy. Aneurysm of left ventricle following infarction. Left descending coronary occluded by thrombus |
| | | 2 | Taken three days after first tracing. Nodal tachycardia | |
| | | 3 | Taken two days after second tracing. Premature contractions. Tendency for fusion of R and T waves in Derivations I and II | |
| | | 4 | Taken day after third tracing and on the day of death. Delayed A-V conduction (P-R 0.24 second). Probably effect of digitalis | |
| 6 (A425425) | M 43 | 1 | Taken two days after admission and before attack. T wave negativity in Derivations II and III | Patient recovered |
| | | 2 | Taken during attack four days after first tracing. Tendency for fusion of R and T waves in Derivations II and III. T wave negativity in Derivations II and III | |
| | | 3 | Taken day after second tracing. T wave negativity in Derivations I and II. T wave diphasic in Derivation III. Fusion of R and T waves has disappeared | |
| | | 4 | Taken day after third tracing. T wave negativity in Derivations I, II and III | |
| | | 5 | Taken day after fourth tracing. T wave negativity in Derivations I, II and III | |

TABLE I—CONT'D

MYOCARDIAL INFARCTION (SERIES OF WILLIUS AND BARNES)

| CASE | SEX AND AGE | TRACING | ELECTROCARDIOGRAM | REMARKS |
|---|---|---|---|---|
| 7 (A448624) | M 67 | 1 | Taken during attack. Ventricular premature contractions. Notched QRS complexes in Derivations I and II (QRS 0.12 second) T wave negativity in Derivations II and III | Necropsy. Multiple infarcts of the left vetricle |
| | | 2 | Taken day after first tracing. Notched QRS complexes in Derivations I and II (QRS 0.12 second), T wave negativity in Derivations I, II and III | |
| | | 3 | Taken two days after second tracing and two days before death. Notched QRS complexes in all Derivations (QRS 0.12 second). T wave negativity in Derivations I and II | |
| 8 (A130266) | F 60 | 1 | Taken forty-six days after attack. T wave negativity in Derivations I, II and III | Patient recovered |
| 9 (A461768) | M 47 | 1 | Taken during attack. Sinus arrhythmia | Patient recovered |
| | | 2 | Taken day after first tracing. Nodal premature contractions | |
| | | 3 | Taken day after second tracing. T wave negativity in Derivation I. Right bundle branch block | |
| | | 4 | Taken day after third tracing. T wave negativity in Derivations II and III | |
| | | 5 | Taken four days after fourth tracing. T wave negativity in Derivation I | |
| | | 6 | Taken nine days after fifth tracing. T wave negativity in Derivation I. Greater amplitude of T wave | |
| | | 7 | Taken twelve days after sixth tracing. T wave negativity in Derivation I. Ventricular premature contractions | |
| | | 8 | Taken four days after seventh tracing and on day of dismissal. T wave negativity in Derivation I. Ventricular premature contractions | |

(89 per cent) cases (Table I). The most frequent change was the negativity of the T wave involving Derivation I, combined Derivations I and II, combined Derivations II and III, and combined Derivations I, II, and III. In three cases (Cases 4, 7 and 9), the derivations involved varied during the period of observation. In two cases (Cases 5 and 6) a tendency for fusion of the R and T wave occurred as a transient manifestation, and quite closely resembled the finding described by Pardee.

Notching and broadening of the QRS complex involving all derivations occurred in only one instance (Case 7), at first affecting only Derivations I and II, but ultimately involving all derivations.

In one case (Case 2) a paroxysm of nodal tachycardia occurred preceding death, and in another case (Case 5) auricular flutter and nodal tachycardia.

The electrocardiogram was normal in only one case (Case 3); it was taken twenty-three days before death and is, therefore, not representative, as possibly alterations would have been noted if other tracings had been obtained. The cases of other observers are recorded in Table II.

SUMMARY OF AGGREGATE SERIES

It is interesting to note that the T wave showed changes in twenty-six of the thirty-one cases (84 per cent, Table III). The alterations consisted of

TABLE II

MYOCARDIAL INFARCTION (CASES COLLECTED FROM THE LITERATURE)

| AUTHOR | CASE | SEX AND AGE | TRACING | ELECTROCARDIOGRAM | REMARKS |
|---|---|---|---|---|---|
| Levine and Trantor | 2 | M 59 | 1* | Taken thirty-six hours after admission. Complete heart block, auricular rate 143, ventricular rate 29 | Necropsy. Left anterior coronary artery was occluded by a thrombus |
| Herrick | 1 | M 42 | 1 | Taken forty-two days after occlusion. Negative T wave in Derivations I and II Left ventricular preponderance | Necropsy. Descending branch of left coronary and first descending branch of the circumflex obstructed by a thrombus |
| | | | 2 | Taken 137 days after first tracing. Negative T wave in Derivation I. Left ventricular preponderance | |
| Pardee | 1 | M 38 | 1 | Taken four hours after attack. Fusion of the R and T waves especially in Derivations II and III. T wave negative in Derivation I, positive in Derivations II and III | |
| | | | 2 | Taken three days after first tracing. Fusion of R and T waves absent. T wave negative in Derivation III | |
| | | | 3 | Taken six days after second tracing. T wave negative in Derivations II and III | |
| | | | 4 | Taken seven days after third tracing, same | |
| | | | 5 | Taken sixty-six days after fourth tracing, same | |
| Robinson and Herrmann | | M 53 | 1 | Taken soon after admission. Ventricular tachycardia, rate 185 | Necropsy. Obstruction of anterior descending branch of the left coronary |
| | | | 2 | Taken ten hours after first tracing. Sinus rhythm, negative T wave in Derivation I | |
| Kahn | 1 | M 51 | 1 | Taken fifty-one days after attack. Nodal premature contractions. Negative T wave in Derivation I, broadening of the QRS in all derivations, 0.12 second. Left ventricular preponderance | Necropsy. Nearly complete calcareous obstruction of the left coronary. An area of softening about 2 cm. in circumference at the apex of the left ventricle |
| | | | 2 | Taken sixty-seven days after first tracing and a few days before death. Diphasic T wave in Derivation I, QRS 0.10 to 0.12 second. Left ventricular preponderance | |
| | 2 | F 46 | 1 | Taken thirty-nine days after occlusion. Negative T wave in Derivation I, notched QRS in Derivation II. Left ventricular preponderance | Necropsy. Occlusion of anterior descending branch of the left coronary |
| | | | 2 | Taken 113 days after first tracing, one day before death. Negative T wave in Derivation I, but less amplitude. Notched QRS in Derivation II, left ventricular preponderance more marked | |

TABLE II—CONT'D

MYOCARDIAL INFARCTION (CASES COLLECTED FROM THE LITERATURE)

| AUTHOR | CASE | SEX AND AGE | TRACING | ELECTROCARDIOGRAM | REMARKS |
|---|---|---|---|---|---|
| Reznikoff | 1 | M 43 | 1* | Alternate slowing and acceleration of rate in units of about 8 beats. Interpreted by author as either a phenomenon of two foci, a normal sinus and a focus with a slower rate near the sinus, or an alternating bradycardia and tachycardia from the same focus | Necropsy. Thrombosis in the left coronary artery and an irregular laceration in the wall of the left ventricle in immediate proximity to the interventricular septum, midway between the apex and base |
| Wearn | 1 | | 1* | Taken four days after onset of attack and two days before death. Auricular premature contractions, negative T waves in Derivations II and III | |
| | 2 | | 1* | Taken two days after onset of attack and on the day of death. Complete heart block, QRS spread in all derivations, negative T wave in Derivation I, left ventricular preponderance | |
| | 3 | | 1* | Taken nine days after onset of attack and on the day of death. Ventricular premature contractions, T wave isoelectric in all derivations | |
| | 4 | | 1* | Taken fourteen days after onset of attack and ten days before death | |
| | 5 | | 1 to 5* | Rhythm at first irregular, delayed A-V conduction and complete heart block later. The QRS was normal on the fourth, twelfth and fourteenth days with marked lengthening on the sixth and seventh days. On the fourth day the T wave was diphasic in Derivation I, isoelectric in Derivation II and negative in Derivation III. On the seventh day the T wave was diphasic in Derivation I. On the fourteenth day the T wave was diphasic in Derivations I and II and isoelectric in Derivation III | Patient died on the fourteenth day |
| | 6 | | 1* | Taken three days after onset of attack and on the day preceding death. T wave isoelectric in Derivation III | |
| | 7 | | 1* | Taken day after onset of attack and on the day of death. Ventricular premature contractions, diphasic T wave in Derivation III. Left ventricular preponderance | |
| | 8 | | 1* | Taken one and two days after onset of attack and five and six days before death. Diphasic T wave in Derivation I. The T wave in Derivation II comes off the downstroke of R but on the following day this had returned to normal. Left ventricular preponderance | |
| | 9 | | 1* | Ventricular premature contractions. T wave isoelectric in all derivations, left ventricular preponderance | Onset of attack indefinite |

TABLE II—CONT'D

MYOCARDIAL INFARCTION (CASES COLLECTED FROM THE LITERATURE)

| AUTHOR | CASE | SEX AND AGE | TRACING | ELECTROCARDIOGRAM | REMARKS |
|---|---|---|---|---|---|
| Wearn | 10 | | 1* | Taken twenty-nine days before death. QRS notched in all derivations. Diphasic T wave in Derivation I and negative in Derivations II and III, left ventricular preponderance | |
| Smith | 1 | M 44 | 1 | Taken nineteen days after accident. Negative T wave in Derivations I, II and III. Right ventricular preponderance | Stab wound of heart and ligation of descending branch of left coronary artery in operative repair. Patient recovered |
| | | | 2 | Taken seventeen days after first tracing. Negative T wave in Derivations I, II and III but less amplitude than in first tracing. Right ventricular preponderance | |
| | | | 3 | Taken twenty-three days after second tracing. Negative T wave in Derivations I, II and III, less amplitude | |
| | | | 4 | Taken eight and one-half months after the accident. Diphasic T wave in Derivation III | |
| | 2 | M 51 | 1 | Taken six hours after onset of attack. Negative T wave in Derivation I | The patient recovered |
| | | | 2 | Taken the day after first tracing. Negative T wave in Derivation I with much greater amplitude. The T wave in Derivation II almost isoelectric | |
| | | | 3 | Taken five hours after second tracing. Negative T wave in Derivations I and II | |
| | | | 4 | Taken day after third tracing. Negative T wave in Derivation I, small positive T wave in Derivation II | |
| | | | 5 | Taken day after fourth tracing, same | |
| | | | 6 | Taken day after fourth tracing, same | |
| | 3 | M 39 | 1 | Taken twenty-four hours after onset of attack. Negative T wave in Derivation I | Patient died twenty-one days after occlusion. Necropsy. Occlusion of descending branch of left coronary and two small branches of circumflex |
| | | | 2 | Taken three days after first tracing. Diphasic T wave in Derivation III | |
| | | | 3 | Taken eleven days after second tracing. Negative T wave in Derivations I and II | |
| | 4 | M 42 | 1 | Taken twelve days after attack. Negative T wave in Derivation III | Patient recovered |
| | | | 2 | Taken thirty-three days after first tracing. T wave isoelectric in all derivations | |
| | | | 3 | Taken eleven and one-half months after attack. T wave isoelectric in Derivation I | |
| | 5 | M 63 | 1 | Taken three weeks after attack. Negative T wave in Derivations I, II and III | Necropsy. Descending branch of left coronary occluded |
| | | | 2 | Taken six months after first tracing. Negative T wave in Derivation I | |
| Nathanson 1923 | 1 | M 64 | 1** | Taken during attack. Marked exaggeration of the T wave in all derivations with negativity in Derivations II and III. Left ventricular preponderance | |
| Riesman | 2 | M 45 | 1* | Irregularity in spread of excitation wave in ventricle and a curve compatible with coronary obstruction | |

*Electrocardiograms not published but descriptions given.

**This case is reported through the courtesy of Dr. M. H. Nathanson of Minneapolis, under whose care the observations were made.

TABLE III

SUMMARY OF ELECTROCARDIOGRAPHIC FINDINGS IN THE CASES WITH PUBLISHED TRACINGS OR ACCURATE DESCRIPTIONS (THIRTY-ONE CASES)

| | CASES |
|---|---|
| Changes in the T wave (negative, diphasic and isoelectric curves) in Derivations I; I and II; II and III; I, II and III | 26 |
| Fusion of R and T waves | 4 |
| Complete heart block | 3 |
| Delayed A-V conduction | 1 |
| Aberrant QRS complexes in all derivations | 5 |
| Sinoauricular b'ock | 1 |
| Ventricular tachycardia | 1 |
| Nodal tachycardia | 2 |
| Auricular flutter | 1 |
| Normal | 3 |

negative, diphasic and isoelectric waves in Derivation I, combined Derivations I and II, combined Derivations II and III, and in combined Derivations I, II and III.

Fusion of the R and T wave occurred in four cases, and is such a definite alteration that its presence should always direct attention to the possible existence of acute coronary obstruction. This finding has also been observed during graphic study of the human heart at the time of death.

Complete heart block was observed in three cases. Aberration of the QRS complex in all derivations occurred in five cases. The other findings, delayed A-V conduction, sinoauricular block, ventricular tachycardia, nodal tachycardia and auricular flutter, occurred with such infrequency as to be unimportant. It is significant, however, that there are only three normal electrocardiograms (10 per cent).

The frequency of T wave changes in myocardial infarction we believe is significant, and these findings, in the presence of a suggestive clinical syndrome, will undoubtedly increase the frequency of correct clinical diagnosis. It is hoped that further reports of cases will appear in the literature, as a larger study will obviously be valuable.

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# STUDIES ON THE PHYSICAL AND COLLOIDAL CHEMISTRY OF ARSPHENAMINE

## Part II. The Alkalinized Solution of Arsphenamine*

By A. Stuart Hunter and W. A. Patrick, Baltimore, Md.

IN the previous paper the determination of an isoelectric point of arsphenamine, as indicated by conductivity measurements, was described. Such a minimum conductivity was found when about 2.8 c.c. of 0.1 NaOH per 0.1 gm. sample had been added to a solution of the dihydrochloride. The $P_H$ value at such a point was about 3.39. In an endeavor to approach this minimum from the alkaline side, this investigation was carried over into the consideration of the alkalinized solution. The experimental technic was in all cases the same as has been described in detail.

The first attempt was made using a laboratory preparation of salvarsan sodium made according to the German patent No. 292,149. This was used in the same uniform concentration of 1.0 gm. made up to 200 c.c. A series of solutions were made up with increasing amounts of 0.1 N HCl up to 8.5 c.c. 0.1 N HCl per 0.1 gm. sample. It was surprising to find that there was no *decrease* in conductivity as the acid was added to the salvarsan sodium solution. Instead there was a continual *increase* in a very uniform manner as shown by the curve, salvarsan sodium A on Plate V. Inasmuch as mannitol had been used as a stabilizer in the preparation of this material, it was thought that possibly its presence in some way had prevented the decrease in the specific conductivity of the solution when it was acidified and converted over into the dihydrochloride. This idea was substantiated by the fact that the precipitate in certain of the solutions did not resemble the usual base in physical appearance. The particles seemed to be much more dense; they were not sticky and were a dull cream color instead of the usual bright yellow. Indeed, Aubry and Dermay (1923) claim that definite chemical combination occurs between arsphenamine and certain sugars. They state that an easily hydrolyzed mono- or diglucoside, for example, is formed. Table XII gives the experimental data on the experiments with salvarsan sodium A.

In order to remove the then supposed cause of failure of the specific conductivity to drop during acidification of the disodium salt, a sample of salvarsan sodium was made without mannitol from some base in a manner similar to the patent mentioned. This material when freshly made, dissolved readily in water yielding a bright yellow solution. After drying *in vacuo* for ten days over KOH it was sealed in ampules which accidentally were

*From a dissertation submitted in partial fulfillment of the requirements of the degree of Doctor of Philosophy in the Johns Hopkins University.

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TABLE XII

SALVARSAN SODIUM A (WITH MANNITOL)

| EXP. | C.C. 0.1 N HCL PER 0.1 GM. | SP. C. × $10^5$ | VOLTS | $P_H$ |
|---|---|---|---|---|
| 47 | 0.0 | 21.48 | 0.8774 | 10.65 |
| 48 | 2.12 | 23.12 | 0.8164 | 9.619 |
| 56 | 2.5 | 24.02 | 0.7625 | 8.7070 |
| 58 | 3.0 | 25.48 | 0.5069 | 4.384 |
| 54 | 3.4 | 26.08 | 0.4602 | 3.604 |
| 50 | 4.25 | 28.94 | 0.4390 | 3.235 |
| 51 | 5.0 | 34.50 | 0.4160 | 2.859 |
| 52 | 6.37 | 50.01 | 0.3844 | 2.396 |
| 53 | 8.5 | 84.43 | 0.3569 | 1.846 |

TABLE XIII

SALVARSAN SODIUM B (WITHOUT MANNITOL)

| EXP. | C.C. 0.1 N HCL PER 0.1 GM. | C.C. 0.1 N NaOH PER 0.1 GM. | SP. C. × $10^4$ | VOLTS | $P_H$ |
|---|---|---|---|---|---|
| 117 | 0.0 | 0.0 | 12.19 | 0.8200 | 9.68 |
| 118 | 0.0 | 1.0 | 13.37 | 0.7840 | 9.071 |
| 119 | 0.0 | 2.0 | 15.07 | 0.6832 | 7.366 |
| 110 | 0.0 | 4.0 | 20.87 | 0.4525 | 3.463 |
| 121 | 0.0 | 5.0 | 27.34 | 0.4220 | 2.947 |
| 122 | 0.0 | 6.5 | 38.12 | 0.4013 | 2.501 |
| 123 | 0.0 | 8.0 | 55.50 | 0.3748 | 2.149 |
| 124 | 1.0 | 0.0 | 16.31 | 0.8553 | 10.28 |
| 125 | 3.0 | 0.0 | 24.41 | 0.8983 | 11.01 |
| 126 | 4.0 | 0.0 | 32.01 | 0.9158 | 11.40 |

Plate V. gives the curve for this series.

filled with carbon dioxide. When an examination was made of the material one week later it was found to be almost completely insoluble, yet the uniform concentration gave to the solution a $P_H$ of 9.68. It was observed, however, that this material went into solution readily upon the addition of sufficient acid or alkali, and the conductance increased in either case.

Table XIII gives the data on this series without mannitol.

This alteration in the drug, illustrated plainly the relative instability of the disodium salt of arsphenamine even in the solid phase. The carbon dioxide had evidently converted a part of the material into the base; this would explain the partial solubility of the preparation and its subsequent dissolving upon the addition of either acid or base. Measurements were made on a sample of imported "salvarsan natrium." It was found to have a specific conductivity of 22.48 x $10^4$ mhos and a $P_H$ value of 11.27 as against 21.48 x $10^4$ and 10.65, respectively, the constants for the material which had been prepared according to the designated German patent. The fact that both of these preparations give a clear solution at a $P_H$ value lower than of a freshly alkalinized dihydrochloride solution is another manifestation of the physical difference of the arsphenamine in the two solutions.

The next attempt to locate a point of minimum conductance from the alkaline side resulted in the use of the materials upon which measurements had been made from the acid side. (Part I.) Consequently, the uniform concentration of 1.0 gm. sample was alkalinized and from it a series of solutions which contained increasing amounts of HCl. Table V gives the data on a typical series.

In view of making the comparison more marked, the information obtained from this data is given in the curves of Type III together with the results of analogous experiments made on the same material during its alkalinization. Plate III-C illustrates the conductance changes in a solution of arsphenamine C during its alkalinization and subsequent acidification back to the dihydrochloride. Inasmuch as a break in the alkalinization curve had occurred after about 2.8 c.c. 0.1 N NaOH had been added per 0.1 gm. sample

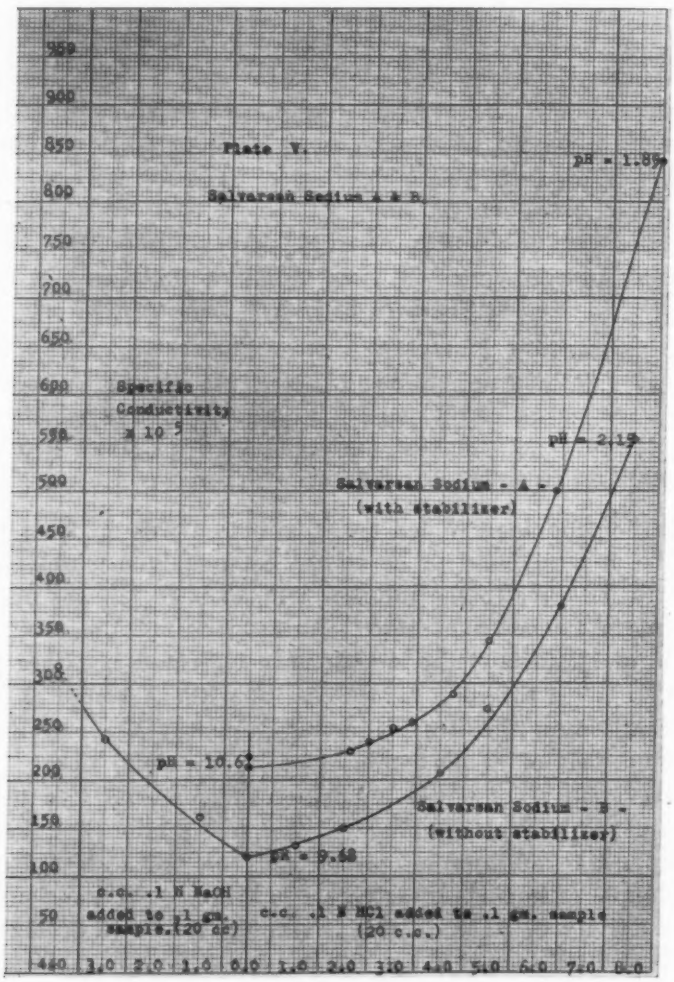
Plate V.
Salvarsan Sodium A. K. P.
pH = 1.89
Specific Conductivity x 10 5
pH = 2.19
Salvarsan Sodium - A -
(with stabilizer)
pH = 10.6
Salvarsan Sodium - B -
(without stabilizer)
pH = 9.68
c.c. .1 N NaOH added to .1 gm. sample.(20 c.c.)
c.c. .1 N HCl added to .1 gm. sample (20 c.c.)

Plate V.

of the dihydrochloride, it might be expected that a similar break might occur after about 5.7 c.c. 0.1 N HCl had been added to the same alkalinized solution. Such was not the case. Instead, a minimum point did occur in the case of arsphenamine C when 1 c.c. 0.1 N HCl had been added; and, from that point the conductance increased continuously. That this phenomena was general is evidenced by Plate III-K which gives the results of the experiments conducted on the six different commercial preparations examined. It must be noticed that in the cases of arsphenamines A, B, C, G, and I that

the break came when 1.0 c.c. acid had been added, while with arsphenamines D, E, F and H the minimum was found only after 2.0 c.c. acid had been added. Table XV shows the relation between the increases in conductance of the different preparations upon alkalinization and acidification.

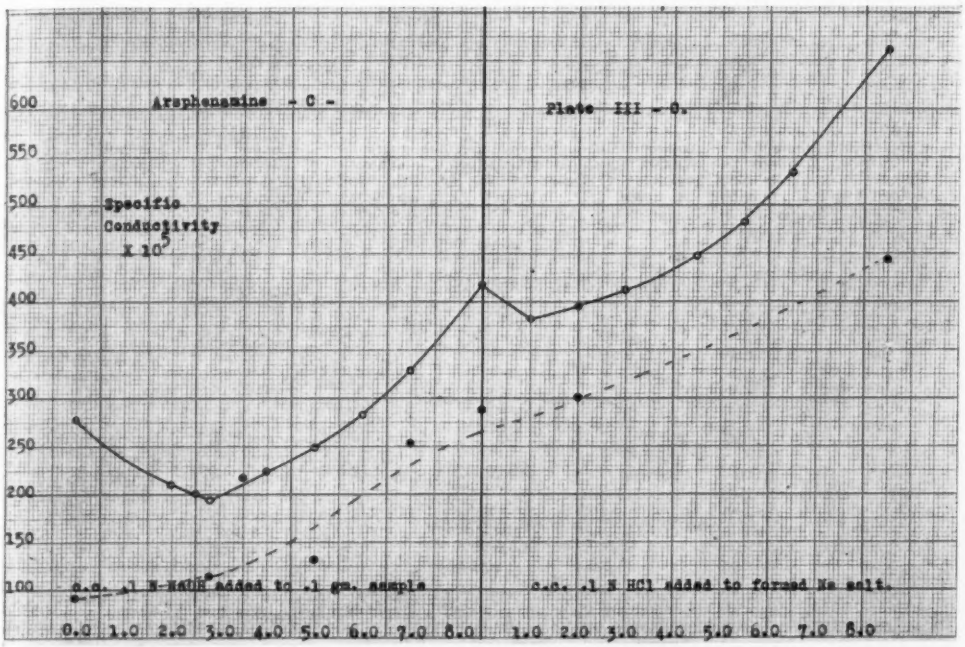


Plate III-C.

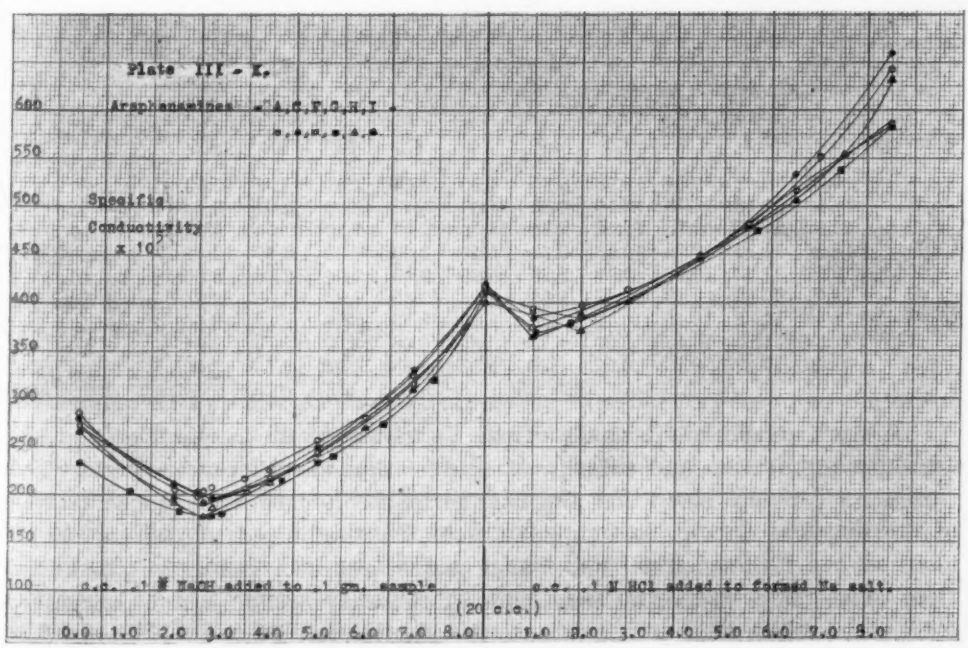


Plate III-K.

TABLE V

ARSPHENAMINE C (ACIDIFICATION OF ALKALINIZED SOLUTION)

| EXP. | C.C. 0.1 N NaOH PER 0.1 GM. | C.C. 0.1 N HCL PER 0.1 GM. | SP. C. X $10^4$ | VOLTS | $P_H$ |
|---|---|---|---|---|---|
| 245 | 8.5 | 0.0 | 41.78 | 0.9288 | 11.52 |
| 254 | 8.5 | 1.0 | 38.23 | 0.9012 | 11.05 |
| 255 | 8.5 | 2.0 | 39.50 | 0.8834 | 10.75 |
| 256 | 8.5 | 3.0 | 41.25 | 0.8517 | 10.22 |
| 257 | 8.5 | 4.5 | 44.74 | 0.6826 | 7.356 |
| 258 | 8.5 | 5.5 | 48.31 | 0.4691 | 3.743 |
| 259 | 8.5 | 6.5 | 53.46 | 0.4270 | 2.475 |
| 260 | 8.5 | 8.5 | 66.11 | 0.3941 | 3.032 |

Column 2 gives the NaOH used to alkalinize the sample of the dihydrochloride; Column 3, the HCl used in acidification; Column 4, the specific conductivity of the solution; Column 5, potentiometric reading in volts (25° C.) and Column 6, the equivalent PH values.

TABLE XV

COMPARISON OF SPECIFIC CONDUCTIVITY CHANGES

| ARSPHENAMINE | DIHYRO-CHLORIDE | DISODIUM | INCREASE | DIHYDRO-CHLORIDE | INCREASE | TOTAL INCREASE |
|---|---|---|---|---|---|---|
| A | 28.46 | 41.65 | 13.19 | 64.22 | 22.57 | 30.76 |
| B | 28.43 | 34.63 | **10.39** | 60.06 | **21.24** | 31.63 |
| C | 27.82 | 41.78 | 13.96 | 66.11 | 24.33 | 38.29 |
| D | 29.97 | 42.41 | 12.30 | 64.79 | 22.52 | 34.82 |
| E | 30.82 | 42.44 | **11.62** | 65.01 | **22.57** | 34.19 |
| F | 27.01 | 41.03 | 14.02 | 58.48 | 17.45 | 31.47 |
| G | 23.20 | 41.24 | **18.04** | 58.40 | **17.16** | 35.20 |
| H | 27.19 | 39.93 | 12.74 | 64.49 | 24.56 | 37.30 |
| I | 26.88 | 41.96 | 15.08 | 63.40 | 21.44 | 36.52 |

Column 2 gives the specific conductivity of the dihydrochloride solution; column 3, that of the alkalinized solution; column 4, increase upon alkalinization; column 5, specific conductivity of solutions reconverted into the dihydrochloride; column 6, increase due to reconversion and column 7, total increase in going from dihydrochloride to dihydrochloride.

TABLE XVI

COMPARISON OF $P_H$ CHANGES

ACIDIFICATION OF THE ALKALINIZED SOLUTION

| | C.C. 0.1 N HCL ADDED PER 0.1 GM. SAMPLE | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SAMPLE | 0.0 | 1.0 | 2.0 | 3.0 | 4.0 | 5.0 | 6.0 | 7.0 | 8.0 | 8.5 | C.C. 0.1 N HCL AT MINIMUM |
| A | 11.55 | 11.22 | 10.51 | 10.03 | 8.35 | 4.90 | 3.58 | 2.97 | 2.97 | 2.52 | 1.0 |
| B | 11.40 | 11.16 | 10.78 | 10.10 | 8.80 | 4.60 | 3.40 | 2.92 | 2.64 | 2.46 | 1.0 |
| C | 11.52 | 11.15 | 10.75 | 10.22 | 8.75 | 4.78 | 3.40 | 2.90 | 2.60 | 2.47 | 1.0 |
| D | 11.56 | 11.12 | 10.74 | 10.09 | 9.34 | 4.65 | 3.90 | 2.92 | 2.60 | 2.42 | 2.0 |
| E | 11.52 | 11.22 | 10.69 | 9.96 | 9.23 | 5.30 | 3.40 | 2.94 | 2.70 | 2.60 | 2.0 |
| F | 11.51 | 10.89 | 10.45 | 10.07 | 8.85 | 4.50 | 3.40 | 2.95 | 2.68 | 2.58 | 2.0 |
| G | 11.42 | 10.82 | 10.45 | 10.12 | 9.60 | 7.00 | 3.55 | 2.98 | 2.68 | 2.56 | 1.0 |
| H | 11.54 | 11.25 | 10.54 | 9.99 | 9.18 | 5.35 | 3.58 | 3.00 | 2.70 | 2.61 | 2.0 |
| I | 11.56 | 11.27 | 10.82 | 10.09 | 8.70 | 5.05 | 3.48 | 3.00 | 2.70 | 2.60 | 1.0 |

When a comparison is made of the $P_H$ values of the respective solutions during alkalinization and subsequent acidification no differences occur. The curves of Type IV show that the change in the hydrogen-ion concentration of the solutions during the acidification of the alkalinized solution is almost identical but reversed from that change which occurred during the alkalinization. Plate IV-C shows the two sides of the curve to be mirror images of each other. This is of course in accord with theory. Plate IV-K shows that this same relationship holds for the commercial preparations. Arsphenamine

G seems to be somewhat different from the rest in that it exhibits a sharper break at the point of alkalinization.

Table XVI gives a comparison of the $P_H$ changes of the respective solutions during acidification. Although some difference occurs in the middle of the precipitation region, it is not as great a variation as was found in a similar spot during alkalinization. (Part I.) The figures show that as far as chemical reaction goes there is no difference between that occurring in the acid solution or the alkaline solution. On the other hand, the difference in the conductance of the two solutions illustrates most strongly a difference in the physical condition of the arsphenamine molecule within the solutions.

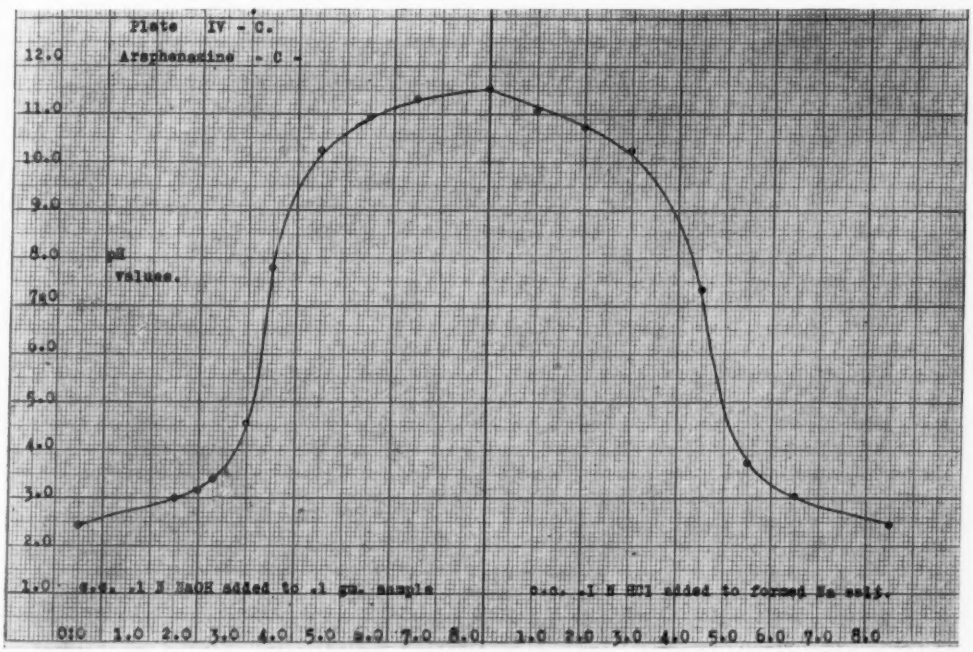


Plate IV-C.

It is to this physical condition that the cause of the toxicity and therapeutic activity must be traced.

In view of the fact that an isotonic saline is recommended by certain clinics (Fordyce, Rosen and Myers, 1923) as a diluent of an alkalinized solution to be used for intravenous injection, it was thought worth while to examine an isotonic arsphenamine solution in order to determine the effect of the electrolyte on the behavior of the arsphenamine molecule in solution. A series of solutions were made up in the regular manner from arsphenamine C and to each the equivalent of 0.85 gm. NaCl was added per 100 c.c. solution. The specific conductivity of 0.85 gm. NaCl in 100 c.c. was determined and taken as a blank. This value when subtracted from the conductances of the respective isotonic arsphenamine solutions gave the conductance of the arsphenamine as such. Now, if these values for the conductance of the arsphenamine, when plotted in the usual manner against

the alkali and acid used, showed any marked deviation from the original curve, such a divergence must have been due to the effect of the excess of electrolyte. Although only preliminary experiments have been completed, a divergence was noted. No minimum was found in the entire curve. Instead, the conductance values increased throughout the alkalinization as well as the acidification.

The conductance of the arsphenamine itself is indicated roughly by the dotted curve on Plate III-C. This abnormal conductivity throughout the entire curve suggests the presence of the colloidal electrolyte in the acid as well as the alkaline solution. Also, macroscopically there was a difference in the solutions which contained a precipitate. There the precipitate is non-

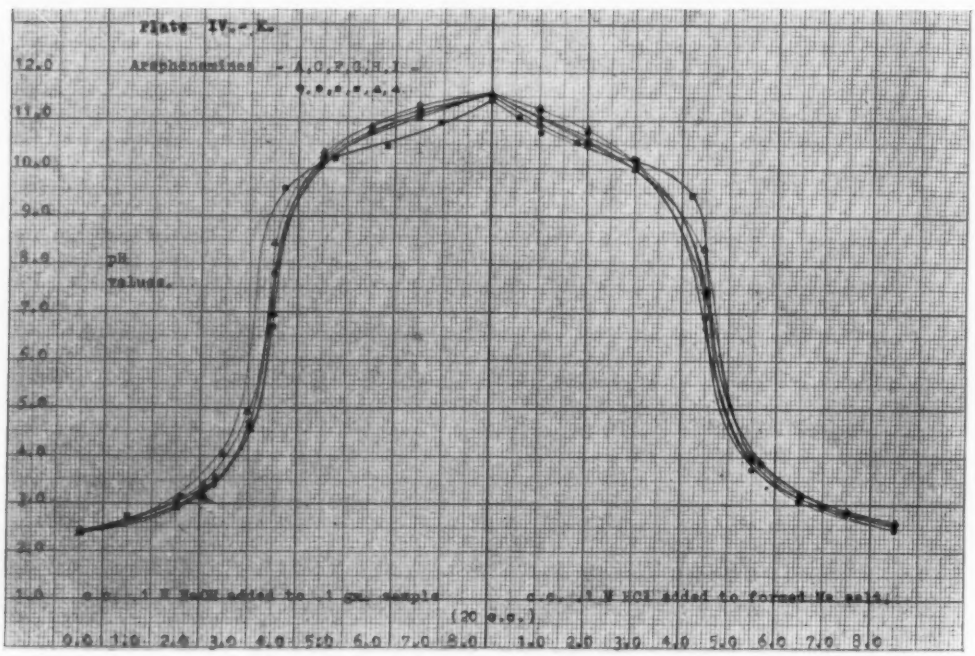


Plate IV-K.

sticky and it tends to form small dense particles in contrast to the gelatinous nature of usual precipitates. This would be expected from colloidal generalizations.

It was observed that upon standing, a sealed, alkalinized solution slowly acquired a red coloration; it became turbid and finally there settled a red gel leaving a colorless supernatant liquor. Gel formation under these conditions required a period of at least three weeks. An attempt was made to ascertain the course of this transformation by following it with conductivity and hydrogen-ion measurements. For this purpose a solution of alkalinized arsphenamine was sealed in 50 c.c. Erlenmeyer flasks which were kept at 25° in an air bath. Plate VI shows how these respective factors changed with time. $P_H$ values dropped slightly, a fact which could not be in accord with an hydrolysis of the sodium salt and the deposition of the base as the

red gel. It was observed that if a turbid solution was exposed to the air for a few minutes and then resealed, that the final supernatant liquor had a green coloration, a phenomena which is often noticed in a solution made up of dry salvarsan sodium. Doubtless, this change is due to some structural polymerization as atmospheric oxidation results in a brownish black residue.

The irregularity of the conductivity curve, the deviations of which are beyond the experimental error, merely emphasizes the complexity of the rearrangement. The turbid solutions poisoned the electrodes and consequently measurements could not be obtained beyond that point.

At present it does not seem possible to correlate the various minor discrepancies which have appeared in the different phases of this investigation. Although it is clearly recognized that the disturbing influences such

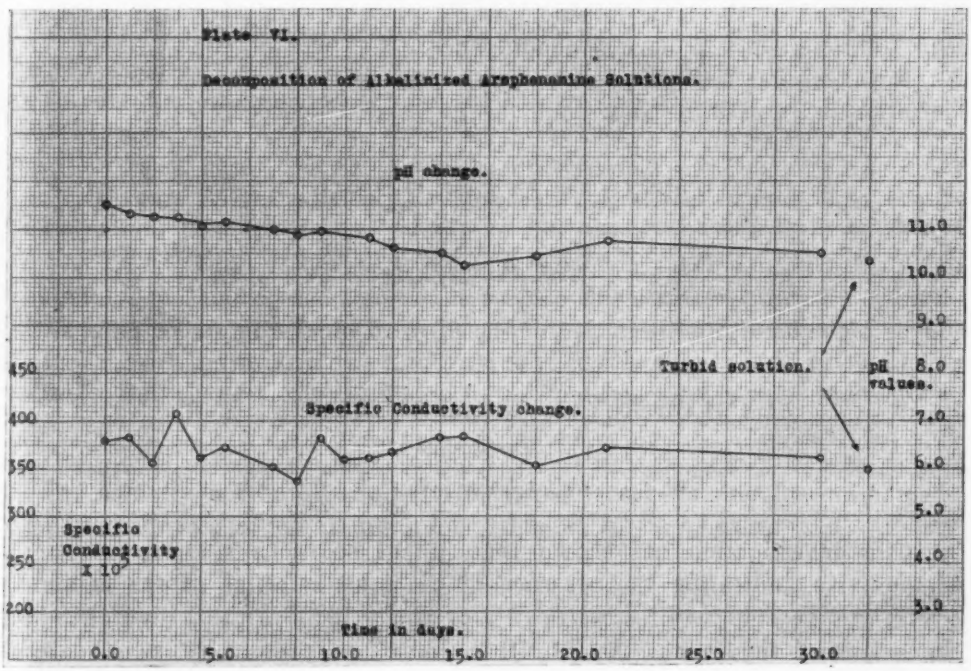


Plate VI.

as hydration, polymerization, etc., might affect the position of the minimum conductivity and consequently shed uncertainty upon the indicated position of the isoelectric point, the fact that the found minimum has been so consistent tends to show that these outside effects have been negligible. Among the arspehnamines examined were preparations of French, German, and American manufacture as well as materials which were very soluble together with those difficultly so. It would seem that if the possible disturbing influences were to make themselves felt that they would have shown up clearly among such an assortment.

The question of the abnormal conductivity merits study, as it is a characteristic phenomena exhibited by the so-called colloidal electrolytes. McBain (1922) has applied the principle of a colloidal electrolyte to soap solutions

which also possess an abnormal conductivity. Soap solutions according to this idea, are supposed to undergo an ionization which might be expressed by the following equilibrium.

$$RNa \rightleftarrows \overset{-}{R} + \overset{+}{Na}$$
$$\uparrow\downarrow -$$
$$(R)_x$$

where the dissociated fatty acid ion R is also in equilibrium with an aggregate of itself which possesses an accumulated charge. This aggregate is termed "ionic micelle" and to it, by virtue of its accumulated charge, is attributed the abnormal conductivity. In the case of soap, i.e., the salts of fatty acids, true solution exists only in great dilutions under which condition little or none of the ionic micelle form is present. Only in such cases do soap solutions show a normal conductance.

Now consider this principle as applied to arsphenamine solutions: The ionization of the sodium salt would be:

$$(AsC_6H_3HN_2O)_2Na_2 \leftrightarrows (AsC_6\overset{- -}{H_3}HN_2O)_2 + 2\ \overset{+}{Na}$$
$$\uparrow\downarrow$$
$$(AsC_6\overset{- -}{H_3}NH_2O)_x$$

On the other hand, the ionization of the dihydrochloride might be expressed:

$$(AsC_6H_3OHNH_3)_2Cl_2 \leftrightarrows (AsC_6\overset{+ +}{H_3}OHNH_3)_2 + 2\ \overset{-}{Cl}$$
$$\uparrow\downarrow$$
$$(AsC_6\overset{+ +}{H_3}OHNH_3)_x$$

This investigation has shown that in the solutions studied, abnormal conductivity, indicating the presence of the ionic micellae of the colloidal electrolyte, became manifest at the $P_H$ of about 10.5. At that hydrogen-ion concentration the anion $(AsC_6H_3NH_2O)$ must be present to some extent in the form of the ionic micelle. Plates III (A-K) show that the conductivity increased in an abnormal manner after the addition of 1.0 or 2.0 c.c. 0.1 N HCl had been added per 0.1 gm. sample to the alkalinized solution. It is possible to conceive that just at those points a sufficient concentration of Na-ions has been built up, due to the NaCl formed by metathesis, to suppress the normal ionization which instead of resulting in the increase of molecular arsphenamine, causes a building up of the ionic micellae. On the other hand, in the isotonic solutions, where there is an excess of the neutral electrolyte present at all times, the ionic micellae are built up at the expense of the true ions. Consequently, instead of a decrease in the conductance upon alkalinization of the hydrochloride there is found the constant increase.

The presence of ionic micellae in arsphenamine solutions might prove an explanation for the paradox presented by the work of Voegtlin and Johnson (1924) on the relationship between the viscosity and the toxicity of the drug.

They found in the hydrochloride solutions that the most viscous were the most toxic while upon alkalinization the most viscous hydrochlorides became the least toxic. This difference in the acid and alkaline solutions could not be explained.

This investigation has pointed out that the colloidal electrolyte, i.e., the ionic micelle form, as indicated by the abnormal conductivity did not make itself manifest in the hypertonic solutions until after such a solution had been alkalinized and its $P_H$ lowered by the addition of acid. A similar drop in the $P_H$ of the alkalinized solution must occur when the solution is injected intravenously, due to the buffer action of the blood. The alkalinized solution then contains the ionic micelle form of the drug. On the other hand, Plates II (A-K) show that as the alkalinization of the hydrochloride proceeds and the $P_H$ value increases, that the conductivity drops suddenly into a region which is characterized by the molecular form of the drug and a subsequent absolute precipitation of the base before the $P_H$ of the blood stream is reached. Undoubtedly the extreme toxicity of the dihydrochloride and the monohydrochloride is because of the fact that, due to the buffer action of the blood, such solutions must pass through this point of minimum conductivity where for the most part only the molecular base can stay in solution. Such a condition would lend itself most readily to the formation of emboli which are always to be found following a fatality caused by such an injection. Undoubtedly, the aggregated ion would be in a physical state much more suitable for its union, possibly with some protein in the blood stream than would be any molecular form of the base. Such a combination would favor longer retention and would lessen the tendency for direct precipitation by electrolytes.

## SUMMARY

1. A study of the alkalinized solution of arsphenamine has been made with the purpose of obtaining information as to its physical and colloidal nature.

2. The conductance of salvarsan sodium solutions does not drop to a minimum when the solutions are acidified to the dihydrochloride; instead it constantly increases.

3. When freshly alkalinized solutions are acidified, the conductivity measurements show a minimum after 1.0 or 2.0 c.c. of 0.1 N HCl have been added per 0.1 gm. sample. No minimum occurs at a $P_H$ value corresponding to that of the isoelectric point found during the alkalinization of the dihydrochloride.

4. $P_H$ measurements show that the changes in the solution upon the acidification of an alkalinized solution are identical and the reverse of those occurring during the alkalinization.

5. Conductance measurements, on the other hand, show that there is a physical difference between the acid and alkaline solutions of arsphenamine. The abnormal conductivity shown in the latter indicates that arsphenamine is a colloidal electrolyte.

6. Observations were made upon the changes which take place in an alkalinized solution upon standing.

7. An isotonic arsphenamine solution shows no minimum conductance either upon alkalinization or subsequent acidification.

8. An explanation is offered for the abnormalities found in the conductivity measurements in the different solutions which is based upon McBain's conception of an ionic micelle found in solutions of colloidal electrolytes. This offers a possible explanation of the differences between the toxicities of the acid and alkaline solutions.

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# A PHARMACODYNAMIC STUDY OF THE ANTHELMINTIC PROPERTIES OF TWO OILS OF CHENOPODIUM*

By A. Richard Bliss, Jr., A.M., Phm.D., M.D., Memphis, Tenn.

## INTRODUCTION

THESE investigations were prompted by the facts that (1) the supply of oil of Chenopodium ("American Oil of Wormseed") is inadequate for human and animal medication, and (2) the resulting high price of the "Maryland Oil of Chenopodium" is consequently preventing the extensive use of the drug in the treatment of domestic animals, a field in which it is much needed.[1]

In 1854 an article by Garrigues[2] described the Southern (U.S.) and the Western (U.S.) Chenopodium plants, and accredited the oils distilled from both varieties with equal anthelmintic properties. During the same year, very shortly after the appearance of this article, another paper[3] was published by a second writer protesting against Garrigues' statements concerning the efficacy of the oil distilled from the Western plants ("Western Oil of Chenopodium"), but offering no proofs of the superiority of the oil distilled from the Southern plants ("Maryland, Baltimore, or Southern Oil of Chenopodium").

The second article referred to is probably responsible for the generally accepted opinion that oil of Chenopodium, conforming to the requirements of the United States Pharmacopoeia,[4] can be produced only from *Chenopodium ambrosioides, var. anthelminticum* cultivated in Carroll County, Maryland. The work of Wirth[5] on an oil distilled from plants grown at the University of

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Michigan Botanical Gardens apparently substantiated this opinion. The conclusion of Wirth and others, that the inferiority of the Western oil of Chenopodium was due to a low Ascaridol content, was apparently based entirely upon the low specific gravity of the Western oil they had obtained.

Schimmel's reports[6] indicate that Ascaridol, which apparently corresponds to the heavier fraction obtained in the distillation of oil of Chenopodium, is the part responsible for the anthelmintic properties of the drug. Although this statement has not been proved and no reasons for the conclusion have been stated, it has been generally accepted. Hall and Hamilton[7] demonstrated that although Ascaridol is anthelmintic, it is also a very active gastrointestinal irritant; while the lighter fraction of the oil is more anthelmintic and decidedly less irritating. Salant and Nelson[8] found Ascaridol 30 per cent more toxic than oil of Chenopodium.

The reports of Schimmel[9] and the investigations of Nelson[10] and of Russell[11] indicate that the failure of Wirth and other workers to obtain a satisfactory oil from plants cultivated in the Middle West was probably due to the faulty methods of distillation employed. Konantz's[12] work substantiates the foregoing statement, for the oils prepared by him from Chenopodium plants cultivated in the Middle West meet the U. S. pharmacopeial specifications.

Although a considerable number of pharmacodynamic investigations of the anthelmintic properties of oil of Chenopodium ("Maryland Oil") have been carried out (particularly by Hall, Hamilton, Widgor, Foster, Wilson, and Salant and coworkers), and while it is probably true that some of the oils used in these investigations were admixtures of the Maryland and the Western oils, up to the present time no pharmacodynamic studies have been made of the pure, unmixed Western oil or of the oil distilled from the wild Chenopodium of the Middle Western states.

## MATERIALS AND METHOD

My pharmacodynamic investigations were carried out on three samples of oil of Chenopodium distilled by Konantz.[13] One of the samples was an oil (S/G 0.966*) distilled from plants which had been shipped from Carroll County, Maryland (referred to in this study as "Maryland Oil of Chenopodium"); a second was a sample of an oil (S/G 0.970) distilled from plants cultivated in Adams County, Illinois, (called "Western Oil of Chenopodium" in this article); and the third was a sample of an oil (S/G 0.964) derived from cultivated wild wormseed plants found in Adams County, Illinois, (the "Wild Western Oil of Chenopodium" hereafter referred to).

Because of the fact that oil of Chenopodium in single therapeutic dose has a decidedly higher efficacy against ascarides than is shown by any other anthelmintic, the common ascaris of the dog was used as the test worm in determining the anthelmintic value of the three oils studied. Other varieties of worms found in the experimental animals were disregarded. Hall and Foster,[14] and Hall[15, 16] established the superiority of oil of Chenopodium as an

*U. S. P. IX requirement: 0.955 to 0.980.

ascaricide in dogs. Their results showed that the oil has no equal for the removal of ascarides, since it will, in the vast majority of cases, remove 100 per cent of these worms present in the dog. Clinical observations have shown that the oil is apparently about as effective, when properly administered, against ascarides in man. Hall and Foster[17] experimentally established its efficacy against ascarides in swine; Hall, Wilson and Wigdor[18] in the horse; and Hall[19] in the cat.

The method employed by the writer was that of Hall and Foster,[20] i.e., the collection of all worms from the feces after the treatment and from the dog postmortem. The standard dose of oil of Chenopodium used throughout the investigations was 0.1 mil per kilo, the amount conclusively established by Hall[21] as being capable of removing all ascarides from the dog in the great majority of cases. The apparatus employed was that advocated by Hall[22] for examining feces for evidences of parasitism. The oils were administered in soft, elastic capsules[23] according to the practical dosage recommended by Hall:[24] viz., 5 minims for dogs weighing ten pounds or less; 10 minims for dogs weighing ten to twenty pounds; 15 minims for dogs weighing twenty to thirty pounds; not to exceed 20 minims for dogs weighing over thirty pounds. One fluidounce (30 mils) of castor oil was given immediately after the oil of Chenopodium. The investigations of Hall and Wigdor[25, 26] and Hall and Hamilton[27] indicate that the administration of castor oil immediately after the oil of Chenopodium is of extreme importance, since it is protective and not merely purgative. Oil of Chenopodium is decidedly

TABLE I

WESTERN OIL OF CHENOPODIUM

| NO. | DOG NO. | WT. IN KILOS | OIL CHEN. IN MINIMS | CASTOR OIL IN MILS | NO. ASCARIDES IN STOOLS | NO. ASCARIDES POSTMORTEM | DIGEST. TRACT | EFFICACY AGAINST ASCARIDES | NO. |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 10 | 15 | 30 | 6 | 0 | Normal | 100% | 1 |
| 2 | 2 | 13 | 15 | 30 | 10 | 0 | Normal | 100% | 2 |
| 3 | 3 | 13 | 15 | 30 | 7 | 0 | Normal | 100% | 3 |
| 4 | 6 | 16 | 20 | 30 | 9 | 0 | Normal | 100% | 4 |
| 5 | 7 | 15 | 20 | 30 | 18 | 0 | Normal | 100% | 5 |
| 6 | 9 | 6 | 10 | 30 | 11 | 0 | Normal | 100% | 6 |
| 7 | 10 | 12 | 15 | 30 | 4 | 0 | Normal | 100% | 7 |
| 8 | 11 | 11.5 | 15 | 30 | 7 | 1 | Normal | 87.5% | 8 |
| 9 | 14 | 8 | 10 | 30 | 21 | 0 | Normal | 100% | 9 |
| 10 | 15 | 14 | 15 | 30 | 2 | 0 | Normal | 100% | 10 |
| 11 | 16 | 14.5 | 20 | 30 | 8 | 0 | Normal | 100% | 11 |
| 12 | 19 | 7.5 | 10 | 30 | 2 | 0 | Normal | 100% | 12 |
| 13 | 21 | 12 | 15 | 30 | 9 | 1 | Normal | 90% | 13 |
| 14 | 23 | 14.5 | 20 | 30 | 14 | 0 | Sl. Inflam. | 100% | 14 |
| 15 | 24 | 15 | 20 | 30 | 35 | 0 | Normal | 100% | 15 |
| 16 | 25 | 15 | 20 | 30 | 1 | 0 | Sl. Inflam. | 100% | 16 |
| 17 | 27 | 12 | 15 | 30 | 23 | 0 | Normal | 100% | 17 |
| 18 | 29 | 9 | 10 | 30 | 8 | 0 | Normal | 100% | 18 |
| 19 | 31 | 15 | 20 | 30 | 6 | 0 | Normal | 100% | 19 |
| 20 | 32 | 9.5 | 10 | 30 | 2 | 0 | Normal | 100% | 20 |
| 21 | 33 | 12 | 15 | 30 | 10 | 2 | Normal | 83% | 21 |
| 22 | 35 | 16 | 20 | 30 | 29 | 0 | Sl. Inflam. | 100% | 22 |
| 23 | 38 | 6 | 10 | 30 | 5 | 0 | Normal | 100% | 23 |
| 24 | 39 | 8 | 10 | 30 | 16 | 0 | Normal | 100% | 24 |

Note 1: Average efficacy against ascarides, 98.35 per cent.
Note 2: Percentage showing slight inflammation, 12.5 per cent.

poisonous, constipating, and a gastrointestinal irritant. Castor oil retards the absorption of the oil of Chenopodium, distributes it over a larger surface of the gastrointestinal mucosa, and promotes elimination. My own observations substantiate the foregoing statements. Salant and Nelson[28] also demonstrated that certain fixed oils are of value in preventing poisoning by oil of Chenopodium.

Each experimental dog, housed in a separate, thoroughly cleaned cage, was given no solid food for twenty-four hours. Early the following morning

TABLE II

WILD WESTERN OIL OF CHENOPODIUM

| NO. | DOG NO. | WT. IN KILOS | OIL CHEN. IN MINIMS | CASTOR OIL IN MILS | NO. ASCARIDES IN STOOLS | NO. ASCARIDES POSTMORTEM | DIGEST. TRACT | EFFICACY AGAINST ASCARIDES | NO. |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 42 | 6 | 10 | 30 | 2 | 0 | Normal | 100% | 1 |
| 2 | 43 | 7.75 | 10 | 30 | 8 | 0 | Normal | 100% | 2 |
| 3 | 44 | 10 | 15 | 30 | 3 | 0 | Normal | 100% | 3 |
| 4 | 45 | 16 | 20 | 30 | 18 | 2 | Sl. Inflam. | 90% | 4 |
| 5 | 48 | 15 | 20 | 30 | 4 | 0 | Normal | 100% | 5 |
| 6 | 51 | 8 | 10 | 30 | 16 | 0 | Normal | 100% | 6 |
| 7 | 52 | 10.5 | 15 | 30 | 26 | 2 | Normal | 93% | 7 |
| 8 | 54 | 11.5 | 15 | 30 | 11 | 0 | Normal | 100% | 8 |
| 9 | 55 | 16 | 20 | 30 | 4 | 0 | Sl. Inflam. | 100% | 9 |
| 10 | 56 | 17 | 20 | 30 | 1 | 0 | Normal | 100% | 10 |
| 11 | 59 | 6.5 | 10 | 30 | 8 | 0 | Normal | 100% | 11 |
| 12 | 62 | 6.75 | 10 | 30 | 2 | 0 | Normal | 100% | 12 |
| 13 | 64 | 9.5 | 10 | 30 | 1 | 0 | Normal | 100% | 13 |
| 14 | 67 | 10 | 15 | 30 | 20 | 0 | Sl. Inflam. | 100% | 14 |
| 15 | 68 | 4.5 | 5 | 30 | 6 | 0 | Normal | 100% | 15 |
| 16 | 69 | 6 | 10 | 30 | 5 | 0 | Normal | 100% | 16 |
| 17 | 71 | 13.5 | 15 | 30 | 8 | 2 | Normal | 80% | 17 |
| 18 | 72 | 12 | 15 | 30 | 12 | 0 | Normal | 100% | 18 |

Note 1: Average efficacy against ascarides, 97.77 per cent.
Note 2: Percentage showing slight inflammation, 16.66 per cent.

TABLE III

MARYLAND OIL OF CHENOPODIUM

| NO. | DOG NO. | WT. IN KILOS | OIL CHEN. IN MINIMS | CASTOR OIL IN MILS | NO. ASCARIDES IN STOOLS | NO. ASCARIDES POSTMORTEM | DIGEST. TRACT | EFFICACY AGAINST ASCARIDES | NO. |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 73 | 12 | 15 | 30 | 22 | 0 | Normal | 100% | 1 |
| 2 | 74 | 15 | 20 | 30 | 12 | 0 | Sl. Inflam. | 100% | 2 |
| 3 | 77 | 6 | 10 | 30 | 3 | 0 | Normal | 100% | 3 |
| 4 | 79 | 7.5 | 10 | 30 | 5 | 0 | Normal | 100% | 4 |
| 5 | 80 | 8.5 | 10 | 30 | 16 | 0 | Normal | 100% | 5 |
| 6 | 81 | 10 | 15 | 30 | 8 | 0 | Normal | 100% | 6 |
| 7 | 82 | 16 | 20 | 30 | 6 | 0 | Sl. Inflam. | 100% | 7 |
| 8 | 84 | 15.5 | 20 | 30 | 1 | 0 | Normal | 100% | 8 |
| 9 | 86 | 14.5 | 20 | 30 | 10 | 2 | Sl. Inflam. | 90% | 9 |
| 10 | 87 | 9 | 10 | 30 | 2 | 0 | Normal | 100% | 10 |
| 11 | 88 | 8 | 10 | 30 | 9 | 0 | Normal | 100% | 11 |
| 12 | 90 | 12.5 | 15 | 30 | 6 | 0 | Normal | 100% | 12 |
| 13 | 91 | 11.75 | 15 | 30 | 8 | 2 | Normal | 80% | 13 |
| 14 | 92 | 6 | 10 | 30 | 3 | 0 | Normal | 100% | 14 |
| 15 | 94 | 7 | 10 | 30 | 8 | 0 | Normal | 100% | 15 |
| 16 | 95 | 10 | 15 | 30 | 9 | 0 | Normal | 100% | 16 |
| 17 | 96 | 13.5 | 15 | 30 | 19 | 0 | Normal | 100% | 17 |
| 18 | 98 | 7.5 | 10 | 30 | 3 | 0 | Normal | 100% | 18 |
| 19 | 102 | 12 | 15 | 30 | 2 | 0 | Normal | 100% | 19 |
| 20 | 104 | 15 | 20 | 30 | 30 | 0 | Normal | 100% | 20 |

Note 1: Average efficacy against ascarides, 98.5 per cent.
Note 2: Percentage showing slight inflammation, 15 per cent.

the oil of Chenopodium and the castor oil were administered. No food was given for at least three hours after the drugs. All feces passed during the following five days were carefully examined for ascarides. At the end of the fifth day the animal was killed (shot), and the alimentary tract from the esophagus to anus was slit and examined for ascarides and lesions.

The results of the study are tabulated in Tables I, II, III, and IV.

## SUMMARY

In Table I there are twenty-four dogs with a total of 267 ascarides; an average of 11+ per dog. Of these worms the treatment with the Western oil of Chenopodium removed 263. The treatment was, therefore, 98.50 per cent effective against ascarides, a value extremely close to the mathematical average efficacy in Note 1, Table I.

There are eighteen dogs in Table II with a total of 161 ascarides; an average of 9– per dog. The treatment with the wild Western oil of Chenopodium removed 155 worms, and was, therefore, 96.27 per cent effective, which is a little less than the mathematical average efficacy in Note 1, Table II.

Table III shows twenty dogs with a total of 192 ascarides; an average of 9.6 per dog. The Maryland Oil of Chenopodium treatment removed 188 of the parasites, and was consequently 97.91 per cent effective, a small fraction less than the mathematical average efficacy in Note 1, Table III.

The foregoing figures are tabulated in Table IV.

TABLE IV

| NO. | OIL | NO. OF DOGS | NO. ASCARIDES IN STOOLS | NO. ASCARIDES POSTMORTEM | PER CENT EFFICACY | NO. |
|---|---|---|---|---|---|---|
| 1 | Western Oil | 24 | 263 | 4 | 98.50 | 1 |
| 3 | Wild Western Oil | 18 | 155 | 6 | 96.27 | 2 |
| 2 | Maryland Oil | 20 | 188 | 4 | 97.91 | 3 |

The average efficacy of the three samples of oil was, therefore, 97.56 per cent.

Examination of the digestive tracts of the animals showed a slight degree of inflammation in three dogs of each of the three series, or a total of nine animals out of sixty-two. This small percentage (14.5 per cent) and the very mild degree of inflammation found indicates that this possible phase of the action of the oils of Chenopodium, in the dosage employed, may be disregarded in the cases of all three oils unless the animal already shows a gastroenteritis. It is interesting to note that practically all the cases showing inflammation were among the heavier dogs which had received a larger dose of the oil. The reader is referred to Hall's study[29] of the lesions due to agents used in killing experimental dogs in anthelmintic investigations.

## CONCLUSIONS

This study has shown that oil of Chenopodium properly distilled from plants cultivated in the Middle Western states is as efficacious in dogs against ascarides as the Maryland oil of Chenopodium. On the basis of the experiments with dogs I venture to state that these findings apply also to man.

I am indebted to W. A. Konantz of Quincy, Ill., for the samples of the oils used in this study; and also to Miss Pearl Waddell, A.B., and to C. H. Menge, A.B., for valuable technical assistance rendered during the investigations.

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# THE LEUCOCYTIC PHASE OF HEMOCLASTIC SHOCK*

By Wm. S. Middleton, M.D., and Ethel Thewlis, Madison, Wis.

SINCE the comprehensive report of Widal, Abrami and Iancovesco,[1] the *crise hémoclasique* has been subjected to renewed scrutiny and criticism. The phenomena constituting this unusual reaction following the ingestion of a glass of milk were definitely held by the group working with Widal to result from a failure of the proteopexic function of the liver. In a word, clinically by a disturbance of the hepatic function in relation to its proper fixation of the split products of protein digestion, a syndrome develops which closely simulates the experimental results of peptone injections or those of a temporary Eck's fistula. To this explanation of a protein responsibility for the resultant leucocytic fall, arterial blood pressure fall and alterations in the coagulability and the refractive index of the blood, most authorities subscribe. Brown[2] has analyzed the clinical value of this procedure and concluded that the observations on the blood pressure and the numerical white cell counts constitute adequate criteria for practical purposes. Feinblatt,[3] in a study of eighty healthy men, firmly established the normal occurrence of a leucocytosis on the ingestion of a glass of milk. This confirmatory observation of the normal reaction lent particular significance to the occurrence of a leucocytic decline under similar conditions.

The *crise hémoclasique* is almost constantly observed in cases of catarrhal cholangitis; and the time-honored belief of an error in fat metabolism in this condition led to an investigation of the element of milk responsible for the depression of the leucocytic reaction in these cases. Widal and his coworkers[1] have reported absolutely negative results from tests in which the carbohydrate and the fatty elements of milk were exhibited alone; whereas casein in amounts corresponding to that contained in a glass of milk gave a prompt and severe reaction.†

Since elsewhere in this review of the subject these workers had urged the qualitative rather than the quantitative nature of the hemoclastic shock, it was deemed advisable to reduce the component elements of milk to smaller quantities retaining their relative proportions. Therefore, while the control of whole milk was kept at 200 gm., the quantity of the component elements was in each case divided by two, anticipating quantitative rather than qualitative alterations in the curves. Casein, butter-fat and lactose were used then in these observations in the amounts found in 100 gm. of whole milk. Lactal-

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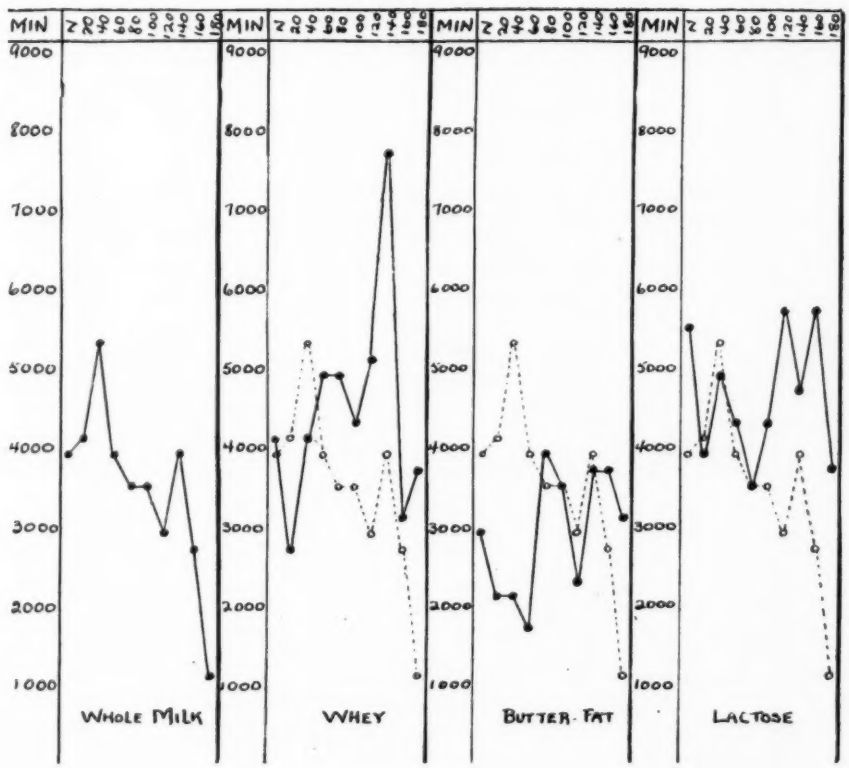
†D'autre part, nous nous sommes assurés que, dans le lait, ce sont bien les substances azotées qui déterminent l'hémoclasie digestive; car chez des sujets qui présentaient après l'ingestion de ce liquide la crise hèmoclasique caractéristique, ni l'absorption d'hydrates de carbone (50 gr. de lactose), ni celle de graisse (30 gr. de beurre essoré), n'ont produit la moindre altération de l'èquilibre hématique. Par contre, après absorption de 8 gr. de caséine sèche, dose correspondent à celle contenue dans 200 gr. de lait, on obtient une réaction très intense et particulièrement précose.

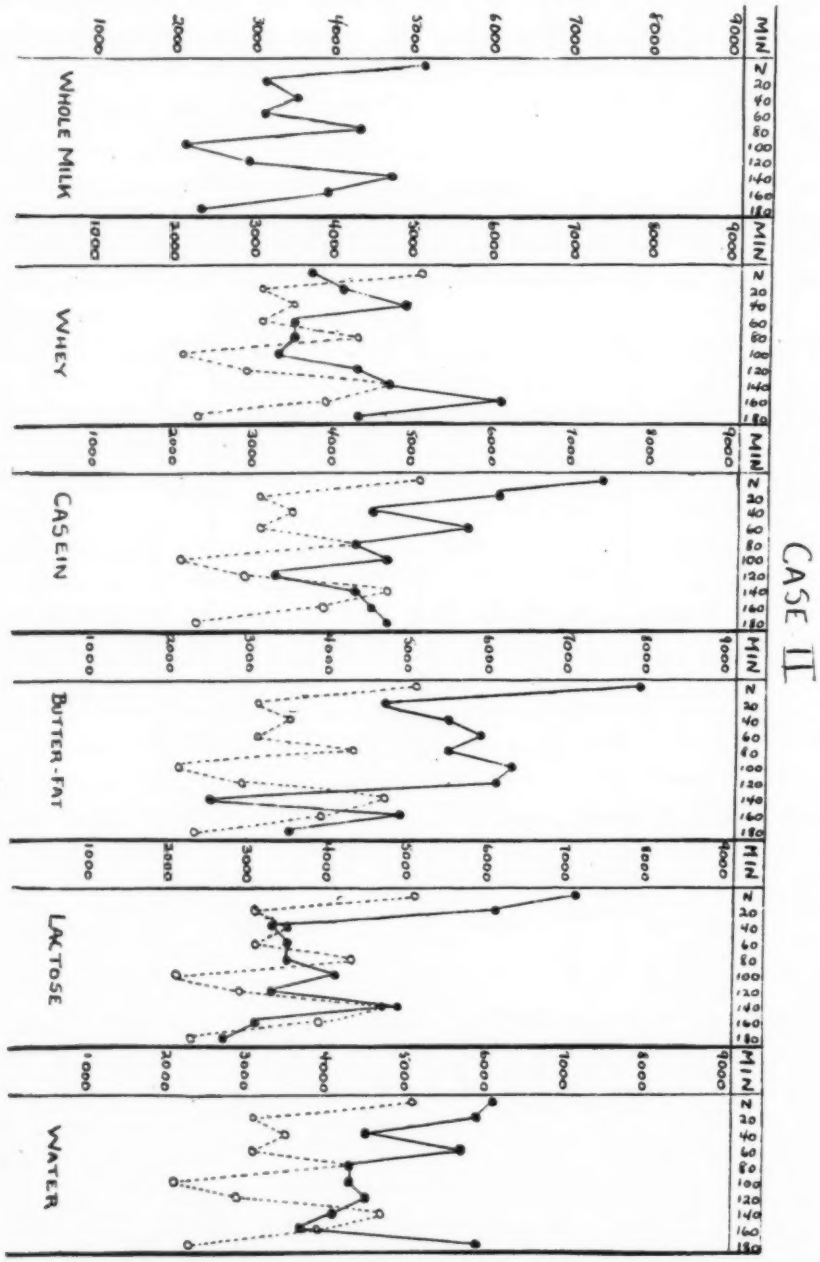
bumin, as such not being available, was given in the form of whey, realizing that lactose in amounts but little below that of whole milk was likewise administered in this procedure. After establishing the normal count in each case, at the same hour, on successive days the several tests were applied. The white cell counts were made every 20 minutes for three hours after the ingestion of the element to be tested. The curves in the three cases followed are appended. The whole milk curve is in each instance drawn in broken lines with the several curves of its component elements in solid lines for comparison.

CASE I



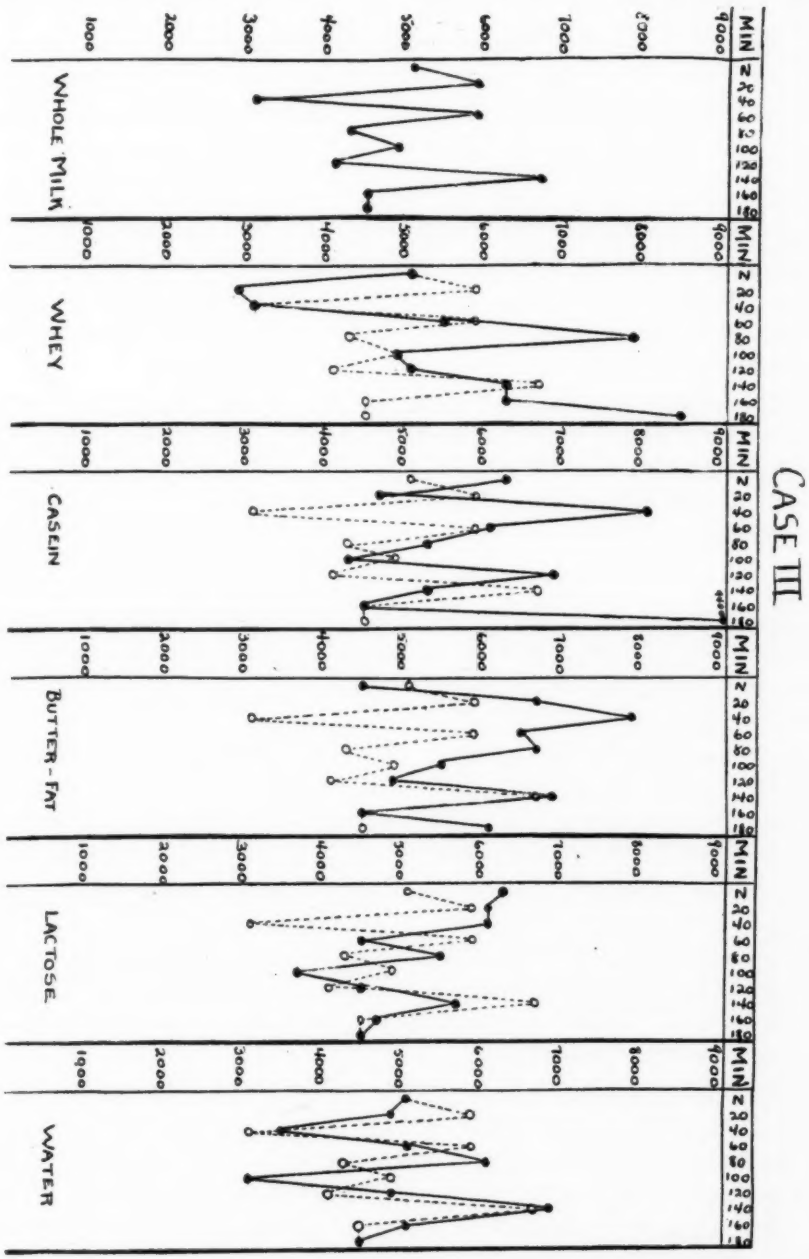

Analysis of these curves reveals certain obvious and illuminating facts. In none has an alimentary leucocytosis succeeded the ingestion of whole milk. The time of appearance of the leucocytic depression is inconstant as is also its degree. Three successive falls in the leucocytic curve may be traced in each instance. Contrary to the results of Widal,[1] casein showed a true shock response in only one of the two cases studied. In only the early phase of Case II does the curve of casein follow that of whole milk. The curves for whey, containing the other protein constituent of milk, are very bizarre, and incapable of reconciliation with their parent curves. Butter-fat on the other hand shows a resemblance to the whole milk curves in two of the three cases. Lactose significantly conforms most closely of all the studied elements of milk to the basic whole milk curve. This concurrence is best

exemplified in Cases II and III, wherein the form of the curves is very similar and only the time element varies. In Case I the degree of fluctuation in the lactose curve is greatly reduced as compared with whole milk, but the form of the curves coincides. The occurrence of a typical reaction on the inges-




tion of water in Case III casts doubt on the significance of the organic factor, protein or otherwise, in the causation of the blood changes. The degree of leucocytic fall with the reduced proportions of the elements of milk as compared with the basic curves of whole milk is surprising in many instances.

Clearly no conclusion can be drawn from such scanty data. However, the indication is clear that a protein element is not essential for the production of hemoclastic shock in cases of catarrhal cholangitis. The whole in-




teresting subject of hemoclastic shock should be critically analyzed before clinical deductions can be accurately drawn.

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# THE DISTRIBUTION OF PHOSPHORUS COMPOUNDS IN THE BLOOD IN TUBERCULOSIS*

By K. Lucille McCluskey, Ph.D., Chicago, Ill.

## I. INTRODUCTION

It is a well established fact that phosphates are indispensable for the growth of the tubercle bacillus. The phosphorus is utilized undoubtedly for the formation of the nucleic acids of the nucleoproteins and the glycerophosphoric acids of the phosphatides, both of which are without question necessary for cell life.

In view of the important rôle of phosphorus in the metabolism of the tubercle bacillus it was considered worth while to study the distribution of phosphorus compounds in the cell and plasma and ascertain if possible, any tendencies toward progressive changes concomitant with the various stages of tuberculosis.

## EXPERIMENTAL

Experimental data were obtained on the corpuscle-plasma ratio, the total phosphorus, the lecithin, the acid soluble, and the inorganic phosphorus of both whole blood and plasma. From the above, the cell content was calculated.

The subjects were male patients from the Municipal Tuberculosis Sanitarium of Chicago. The blood was drawn before breakfast and coagulation prevented by sodium oxalate. The phosphorus determinations were made according to the author's[1] method using trichloracetic acid as the protein precipitant, and the Sundstroem-Bloor[2] technic was used for the hematocrite readings.

The classification of the subjects is the same as that used in an earlier work[3] and is based both upon quantity and type of involvement. The diagrammatic scheme shows graphically the various courses over which the disease may travel. (See Chart I.)

Group VI is considered to be rather transitory and therefore difficult to classify. The early stages of the disease would *a priori* be of the neutral type (i.e., neither the destructive exudative type nor the proliferative healing type, but between these two extremes), with a moderate amount of involvement. This group is designated as III on the chart and represents a condition definitely recognized as tuberculous and first removed from the normal one in this scheme of classification. From Group III the disease may travel over one of three courses, viz.: III to IV; III to II to V; and III to II to I, depending upon the type and quantity of the involvement.

*From the Laboratories of the Municipal Tuberculosis Sanitarium, Chicago, Ill.
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The results of the analyses expressed in mg. of orthophosphoric acid per 100 c.c. of whole blood, plasma, and cells (calculated) are given in Table I. A cytologic study is reported in Table II.

## DISCUSSION

The variations within the groups are no greater than in normal subjects, so that the averages of these various groups are assumed to be representative of the particular condition. All comparative studies are therefore based upon

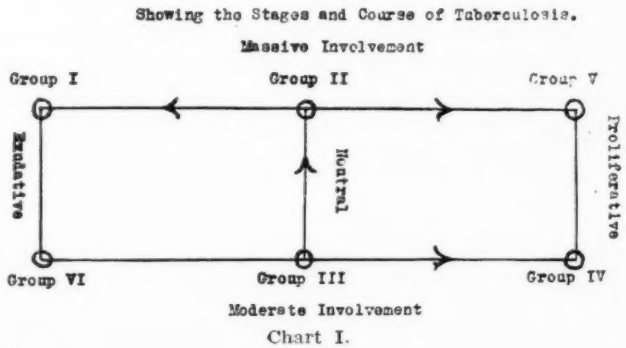


Chart I.

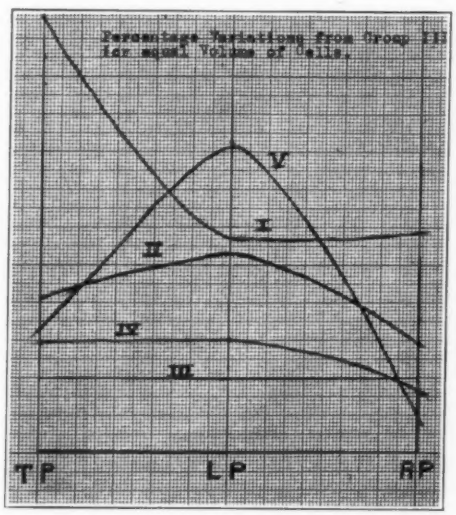


Chart II.

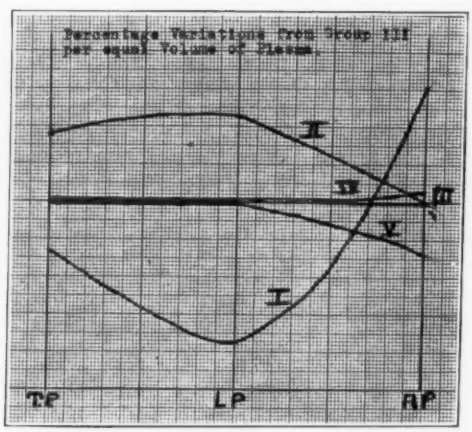


Chart III.

these averages. It is to be regretted that more subjects were not available for Group V, but very few patients of this class remain in a sanitarium.

In order to show changes in the distribution of phosphorus compounds as the disease passes through the various stages, it was thought best to use Group III as the basis for such comparison, since all the other groups have emanated from this particular one. Table III shows the percentage deviations from Group III as calculated from Table I. These percentage differences are graphed in Charts II, III and IV

It is to be borne in mind that these comparisons are based upon equal volumes of corpuscles, plasma, and whole blood (100 c.c.). Equal volumes

of cells do not necessarily contain the same number of cells. The number is a function of the size of the individual cell. Thus a study of changes within the cell should be based upon the content of the individual cell rather than upon unit volume.

That the average cell volume of Group III is about normal is evident if five million red cells, and a corpuscle volume of 45 per cent be taken as the average for the normal male, since the latter figures would give an average cell volume of .90 x 10 – 10 c.c.

From the red cell count, the corpuscle volume, and the phosphorus content per 100 c.c. of cells, the concentrations of the various phosphorus compounds in the cell were calculated and are given in Table IV, together with percentage changes in the size of the cell, in the corpuscle ratio and the phosphorus quotient. The data in the last three columns of the table are expressed in percentage variations from Group III.

TABLE I

PHOSPHORIC ACID CONTENT OF BLOOD OF TUBERCULOUS PATIENTS MG. OF $H_3PO_4$ PER 100 C.C.

| INITIALS AND CASE NO. | SEX | DATE | CLASSIFICATION GROUP | CORPUSCLE PLASMA RATIO | | TOTAL PHOSPHORUS | | | LECITHIN PHOSPHORUS | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | COR. | PL. | WHOLE BLOOD | PLASMA | CELLS (CALC.) | WHOLE BLOOD | PLASMA | CELLS (CALC.) |
| M. L. (17973) | M | 10-29-23 | I | 40.4 | 59.6 | 122.3 | 29.9 | 258.6 | 33.8 | 18.1 | 56.9 |
| F. W. (16891) | M | 12-21-23 | I | 32.0 | 68.0 | 116.6 | 35.6 | 288.7 | 33.5 | 22.2 | 57.5 |
| M. L. (18253) | M | 2-12-24 | I | 33.6 | 66.4 | 146.5 | 35.6 | 365.7 | 31.1 | 21.2 | 67.5 |
| A. O. ( 5654) | M | 2-12-24 | I | 37.1 | 62.9 | 125.0 | 35.0 | 272.2 | 36.7 | 23.9 | 58.4 |
| C. M. (18454) | M | 2-18-24 | I | 40.0 | 60.0 | 149.6 | 33.5 | 323.7 | 33.0 | 19.1 | 53.7 |
| A. M. (18453) | M | 2-18-24 | I | 34.6 | 65.4 | 140.4 | 29.0 | 353.1 | 28.3 | 14.7 | 54.3 |
| AVERAGE GROUP I | | | | 36.2 | 63.7 | 133.6 | 31.1 | 310.3 | 32.7 | 19.8 | 58.0 |
| J. S. (14581) | M | 11-12-23 | II | 47.6 | 52.4 | 132.5 | 39.9 | 234.4 | 40.9 | 29.9 | 53.5 |
| A. J. (16275) | M | 11-12-23 | II | 47.0 | 53.0 | 129.3 | 33.6 | 237.2 | 38.9 | 23.9 | 55.9 |
| J. S. (18036) | M | 1-21-24 | II | 44.7 | 55.3 | 135.3 | 43.6 | 248.7 | 41.5 | 28.5 | 57.7 |
| P. T. (18100) | M | 1-21-24 | II | 42.6 | 57.4 | 137.3 | 33.3 | 277.4 | 34.9 | 18.7 | 56.8 |
| J. O. (18147) | M | 2-21-24 | II | 36.2 | 63.8 | 120.3 | 33.2 | 273.7 | 36.6 | 22.7 | 61.0 |
| A. S. (14579) | M | 10-29-23 | II | 41.4 | 58.6 | 131.1 | 35.6 | 266.4 | 36.3 | 20.4 | 58.6 |
| AVERAGE GROUP II | | | | 43.2 | 56.7 | 130.9 | 36.5 | 256.3 | 38.2 | 24.0 | 57.2 |
| J. O. (17977) | M | 11- 8-23 | III | 38.6 | 61.4 | 134.4 | 36.8 | 289.6 | 39.2 | 25.0 | 61.9 |
| A. H. (18001) | M | 11- 8-23 | III | 43.4 | 56.6 | 117.3 | 31.9 | 229.2 | 32.4 | 21.4 | 46.7 |
| O. J. (17464) | M | 11-22-23 | III | 44.8 | 55.2 | 118.6 | 37.0 | 221.4 | 34.2 | 25.2 | 45.3 |
| F. F. (17924) | M | 11-22-23 | III | 45.8 | 54.2 | 127.5 | 42.6 | 228.1 | 35.7 | 28.7 | 44.1 |
| T. M. (17475) | M | 2-21-24 | III | 44.5 | 55.5 | 111.6 | 30.5 | 212.8 | 35 3 | 20.6 | 53.7 |
| G. N. (18202) | M | 2-25-24 | III | 42.1 | 57.9 | 118.5 | 30.1 | 240.1 | 33.9 | 17.4 | 56.7 |
| F. C. (17592) | M | 2-25-24 | III | 40.8 | 59.2 | 124.1 | 32.8 | 256.6 | 34.0 | 18.8 | 56.1 |
| AVERAGE GROUP III | | | | 42.9 | 57.1 | 121.7 | 34.5 | 239.7 | 34.9 | 22.4 | 52.0 |
| W. F. (16769) | M | 10-24-23 | IV | 46.3 | 53.7 | 131.1 | 35.4 | 241.6 | 37.3 | 25.2 | 51.4 |
| W. C. (17986) | M | 10-24-23 | IV | 43.0 | 57.0 | 119.2 | 30.9 | 236.2 | 33.7 | 17.8 | 54.8 |
| J. G. (18404) | M | 2-25-24 | IV | 38.8 | 61.2 | 117.9 | 41.5 | 238.4 | 35.6 | 27.1 | 48.9 |
| M. L. (18037) | M | 2-25-24 | IV | 42.4 | 57.6 | 117.1 | 34.5 | 229.4 | 34.0 | 22.4 | 49.7 |
| J. M. (17380) | M | 11-28-23 | IV | 44.3 | 55.7 | 119.0 | 32.1 | 228.4 | 37.9 | 22.8 | 56.8 |
| J. C. (18029) | M | 1-21-24 | IV | 39.2 | 60.8 | 139.1 | 32.1 | 305.1 | 35.0 | 18.1 | 61.2 |
| AVERAGE GROUP IV | | | | 42.3 | 57.7 | 123.9 | 34.4 | 246.6 | 35.6 | 22.2 | 53.8 |
| J. W. ( 4130) | M | 12-17-23 | V | 44.2 | 55.8 | 118.6 | 34.0 | 225.5 | 39.9 | 21.2 | 63.5 |
| E. K. (10831) | M | 12-17-23 | V | 41.0 | 59.0 | 132.0 | 34.3 | 272.6 | 42.2 | 20.4 | 68.7 |
| M. J. (13634) | M | 11-22-23 | V | 42.9 | 57.1 | 125.5 | 35.2 | 245.6 | 37.9 | 26.1 | 53.3 |
| AVERAGE GROUP V | | | | 42.7 | 57.3 | 125.3 | 34.5 | 247.9 | 40.0 | 22.5 | 61.8 |

Contrasting Chart II with Chart V, it is evident that a rather erroneous impression of the actual conditions within the cell is obtained if such comparisons are based upon unit volume of cells instead of the cell itself. For this reason a comparison made upon the individual cell is considered preferable since it is the actual condition within the cell itself that is most to be desired.

It would appear from Chart II that the total phosphorus of the cell was increased over Group III in all groups, whereas it is actually less or equal in concentration with the exception of Group I. The same is true of lecithin and similar relations with the acid soluble phosphorus fraction exist.

A summary of Charts III and V indicate that as the disease passes from the moderate involvement neutral type (Group III) to the moderate involvement proliferative type (Group IV) the only change from Group III is a decrease in acid soluble phosphorus in the cell and a slight increase in the

TABLE I—CONT'D

PHOSPHORIC ACID CONTENT OF BLOOD OF TUBERCULOUS PATIENTS MG. OF $H_3PO_4$ PER 100 C.C.

| ACID SOLUBLE PHOSPHORUS | | | LECITHIN PLUS ACID SOLUBLE PHOSPHORUS | | | TP—(ASP PLUS LP) | | | INORGANIC PHOSPHORUS | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| WHOLE BLOOD | PLASMA | CELLS (CALC.) | WHOLE BLOOD | PLASMA | CELLS | WHOLE BLOOD | PLASMA | CELLS | WHOLE BLOOD | PLASMA | CELLS (CALC.) |
| 89.1 | 11.3 | 204.4 | 129.9 | 29.0 | 261.3 | − 0.6 | +0.9 | − 2.7 | 17.8 | 10.6 | 28.9 |
| 76.2 | 13.6 | 209.3 | 109.7 | 35.8 | 266.8 | + 6.8 | −0.2 | +21.9 | 13.2 | 13.0 | 13.7 |
| 102.0 | 14.1 | 218.5 | 133.1 | 35.3 | 346.0 | +13.4 | +0.3 | +19.7 | 19.5 | 12.5 | 33.3 |
| 86.8 | 14.5 | 209.1 | 123.5 | 38.4 | 267.5 | + 1.5 | −0.1 | + 4.7 | 19.3 | 13.7 | 31.5 |
| 106.9 | 13.2 | 247.5 | 139.9 | 32.3 | 301.2 | + 9.7 | +1.2 | +22.5 | 10.2 | 11.3 | 8.5 |
| 102.6 | 14.1 | 271.5 | 130.9 | 28.8 | 325.8 | + 9.5 | +0.2 | +27.3 | 11.4 | 14.1 | 2.9 |
| 92.9 | 13.4 | 226.7 | 126.6 | 33.2 | 294.7 | + 6.7 | +0.4 | +15.5 | 15.2 | 12.5 | 19.8 |
| 92.7 | 12.4 | 181.1 | 133.6 | 41.9 | 234.6 | − 1.1 | −2.0 | − 0.2 | 13.5 | 12.0 | 1.3 |
| 92.1 | 11.7 | 182.7 | 131.0 | 35.6 | 238.6 | − 1.7 | −2.0 | − 1.4 | 14.9 | 11.5 | 18.7 |
| 92.9 | 13.1 | 191.7 | 134.4 | 41.6 | 249.4 | + 0.9 | +2.0 | − 0.7 | 13.4 | 11.7 | 15.4 |
| 100.7 | 14.0 | 217.6 | 135.6 | 32.7 | 274.4 | + 1.7 | +0.6 | + 3.0 | 14.4 | 11.5 | 18.3 |
| 84.3 | 10.8 | 213.8 | 120.9 | 33.5 | 213.8 | − 0.6 | −0.3 | − 1.1 | 8.8 | 10.9 | 5.2 |
| 94.4 | 11.3 | 212.0 | 130.7 | 31.7 | 271.1 | + 0.4 | +3.9 | − 4.2 | 20.3 | 11.0 | 33.5 |
| 92.8 | 12.2 | 199.8 | 131.0 | 36.1 | 246.9 | + 0.0 | +0.3 | − 0.7 | 14.2 | 11.4 | 15.4 |
| 95.6 | 12.2 | 222.8 | 134.8 | 37.2 | 284.7 | − 0.4 | −0.4 | + 4.9 | 12.5 | 11.0 | 15.0 |
| 87.9 | 12.1 | 186.8 | 120.3 | 33.5 | 233.5 | − 3.0 | −1.6 | − 4.3 | | 11.1 | |
| 86.7 | 12.3 | 221.4 | 120.9 | 37.5 | 221.4 | − 2.3 | −0.5 | + 0.0 | | | |
| 91.2 | 12.0 | 184.9 | 126.9 | 40.7 | 229.0 | + 0.6 | +1.9 | − 0.9 | 17.7 | 11.0 | 15.2 |
| 76.0 | 10.8 | 158.6 | 111.3 | 30.3 | 212.3 | + 0.3 | +0.2 | + 0.5 | 7.6 | 9.9 | 4.9 |
| 85.3 | 13.1 | 184.5 | 119.2 | 30.5 | 184.5 | − 0.7 | −0.4 | − 1.1 | 17.6 | 14.6 | 21.8 |
| 88.9 | 13.4 | 197.5 | 122.9 | 32.9 | 253.6 | + 1.2 | −0.1 | + 3.0 | 17.9 | 14.5 | 22.7 |
| 87.3 | 12.2 | 193.8 | 122.3 | 34.6 | 231.3 | − 0.6 | −0.1 | + 0.3 | 14.6 | 12.0 | 15.9 |
| 93.7 | 11.6 | 188.9 | 131.0 | 36.8 | 240.3 | − 0.1 | −1.4 | + 1.3 | 14.2 | 9.0 | 20.3 |
| 86.2 | 13.6 | 182.3 | 119.9 | 31.4 | 237.1 | − 0.7 | −0.5 | − 0.9 | 16.5 | 11.0 | 23.7 |
| 81.2 | 13.4 | 188.1 | 116.8 | 40.5 | 237.0 | + 1.1 | +1.0 | + 1.4 | 17.8 | 11.8 | 27.3 |
| 81.7 | 12.4 | 175.9 | 115.7 | 34.8 | 225.6 | + 1.4 | −0.3 | + 3.8 | 16.5 | 12.6 | 21.6 |
| 79.4 | 9.9 | 166.8 | 117.3 | 32.7 | 223.6 | + 1.7 | −0.6 | + 4.8 | 13.1 | 9.9 | 21.2 |
| 104.5 | 14.8 | 243.6 | 139.5 | 32.9 | 304.8 | − 0.4 | −0.8 | + 0.3 | 15.8 | 12.4 | 21.1 |
| 87.8 | 12.6 | 190.9 | 123.3 | 34.8 | 244.7 | + 0.5 | −0.4 | + 1.8 | 15.6 | 11.1 | 22.5 |
| 80.6 | 11.6 | 167.8 | 120.5 | 32.8 | 231.3 | − 1.9 | +1.2 | − 5.8 | 12.8 | 11.8 | 14.0 |
| 90.0 | 12.4 | 201.7 | 132.2 | 32.8 | 270.4 | − 0.2 | +1.5 | + 2.2 | 15.7 | 11.9 | 21.2 |
| 86.7 | 11.1 | 187.6 | 124.6 | 37.2 | 240.9 | + 0.9 | −2.0 | + 4.7 | 18.3 | 11.0 | 27.9 |
| 85.8 | 11.7 | 185.7 | 125.7 | 34.3 | 247.5 | − 0.4 | +0.2 | + 0.3 | 15 6 | 11.5 | 21.0 |

TABLE II

| INITIALS AND CASE NO. | SEX | AGE | GROUP | RED CELL COUNT | VOLUME PER CENT OF CELLS | CELL VOLUME × $10^{-10}$ (C.C.) | LEUCOCYTES | REMARKS |
|---|---|---|---|---|---|---|---|---|
| M. L. (17973) | M | 38 | I | 4.73 MM | 40.4 | 0.85 | 7.3 M | |
| F. W. (16891) | M | 30 | I | 4.65 | 32.0 | 0.68 | 13.9 | Patient died 10 days later |
| M. L. (18253) | M | 34 | I | | 33.6 | | | Patient died 24 hours later |
| A. O. ( 5654) | M | 33 | I | 4.54 | 37.1 | 0.81 | 11.0 | |
| C. M. (18454) | M | 31 | I | 4.93 | 40.0 | 0.81 | 8.6 | Patient died 5 days later |
| A. M. (18453) | M | 30 | I | | 34.6 | | | Patient died 24 hours later |
| AVERAGE GROUP I | | 32 | | 4.71 | 36.2 | 0.79 | 7.7 | |
| J. S. (14581) | M | 49 | II | 5.85 | 47.6 | 0.81 | 9.3 | |
| A. J. (16275) | M | 21 | II | 4.94<br>5.46 | 47.0 | 0.95 | 8.9 | Patient died 4 months later |
| J. S. (18036) | M | 21 | II | | 44.7 | 0.82 | 9.8 | |
| P. T. (18100) | M | 31 | II | 4.84 | 42.6 | 0.88 | 14.5 | |
| J. O. (18147) | M | 37 | II | 4.98 | 36.2 | 0.72 | 9.9 | |
| A. S. (14579) | M | 36 | II | 4.97 | 41.4 | 0.83 | 8.8 | |
| AVERAGE GROUP II | | 30 | | 5.17 | 43.2 | 0.83 | 10.2 | |
| J. O. (17977) | M | 27 | III | 4.82 | 38.6 | 0.80 | 10.6 | |
| A. H. (18001) | M | 27 | III | 4.47 | 43.4 | 0.97 | 10.6 | |
| O. J. (17464) | M | 26 | III | 5.13 | 44.8 | 0.87 | 7.1 | |
| F. F. (17924) | M | 25 | III | 4.23 | 45.8 | 1.08 | 9.6 | |
| T. M. (17475) | M | 38 | III | 4.60 | 44.5 | 0.97 | 8.2 | |
| G. N. (18202) | M | 19 | III | 4.81 | 42.1 | 0.87 | 8.5 | |
| F. C. (17592) | M | 27 | III | 4.76 | 40.8 | 0.85 | 14.0 | |
| AVERAGE GROUP III | | 27 | | 4.69 | 42.9 | 0.91 | 9.8 | |
| W. F. (16769) | M | 27 | IV | 4.97 | 46.3 | 0.93 | 8.9 | |
| W. C. (17986) | M | 34 | IV | 5.00 | 43.0 | 0.86 | 7.8 | |
| J. G. (18404) | M | 68 | IV | 4.84 | 38.8 | 0.80 | 7.8 | |
| M. L. (18037) | M | 31 | IV | 4.76 | 42.4 | 0.89 | 5.6 | |
| J. M. (17380) | M | 38 | IV | 4.33 | 44.3 | 1.02 | 7.5 | |
| J. C. (18029) | M | 38 | IV | 4.98 | 39.2 | 0.79 | 10.5 | |
| AVERAGE GROUP IV | | 39 | | 4.81 | 42.3 | 0.88 | 8.0 | |
| J. W. ( 4130) | M | 53 | V | 5.03 | 44.2 | 0 83 | 9.5 | |
| E. K. (10831) | M | 40 | V | 4.60 | 41.0 | 0.89 | 11.6 | |
| M. J. (13634) | M | 39 | V | 4.85 | 42.9 | 0.88 | 10.4 | |
| AVERAGE GROUP V | | 44 | | 4.83 | 42.7 | 0.86 | 10.5 | |

TABLE III

PERCENTAGE VARIATIONS FROM GROUP III PER 100 C.C.

| GROUP | TOTAL PHOSPHATE | | | LECITHIN PHOSPHATE | | | ACID SOLUBLE PHOSPHATE | | |
|---|---|---|---|---|---|---|---|---|---|
| | CELLS | PLASMA | WHOLE BLOOD | CELLS | PLASMA | WHOLE BLOOD | CELLS | PLASMA | WHOLE BLOOD |
| I | +29.4 | −4.0 | +9.7 | +11.5 | −11.6 | − 6.3 | +11 8 | +9.8 | +6.4 |
| II | + 6.9 | +5.7 | +7.5 | +10.0 | + 7.0 | + 9.4 | + 3.0 | 0.0 | +6.3 |
| IV | + 2.8 | 0.0 | +1.8 | + 3.0 | 0.0 | + 2.0 | − 1.0 | +1.0 | 0.0 |
| V | + 3.4 | 0.0 | +2.9 | +18.8 | 0.0 | +14.5 | − 3.0 | −4.0 | −2.0 |

plasma. Group II contains more lecithin in the plasma and about the same in the cell as Group III with a decrease in acid soluble phosphorus in the cell. Group V is characterized by a considerable increase in lecithin content in the cell, with a decrease in acid soluble phosphorus in both cell and plasma. It is to be noted that the lecithin is increased in the plasma in Group II. Since

Group II lies next to either extreme (Groups I and V) it is rather difficult to speculate on the cause of this increase. It might represent the passage of lecithin from the cell to the plasma as would be expected in going from Group II to Group I, or an accumulation of lecithin in the plasma preparatory to entering the cell in passing from Group II to Group V. Group I is quite the opposite of Group V with a low lecithin content in both the cell and the plasma, with a high increase in the acid soluble phosphorus in the plasma. The total phosphorus of the cell in Group I is high, though both the lecithin and the acid soluble phosphorus are depressed. This is explained by the presence of a phosphorus fraction in the cell which is not included in the phosphatides or acid soluble groups.

It is to be noted in Table I, Group I, that there is a difference between the total phosphorus and the sum of the lecithin and acid soluble fractions in the whole blood and the cells, but not in the plasma, indicating that this

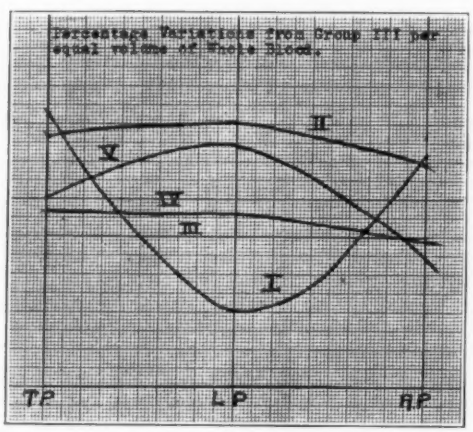


Chart IV.

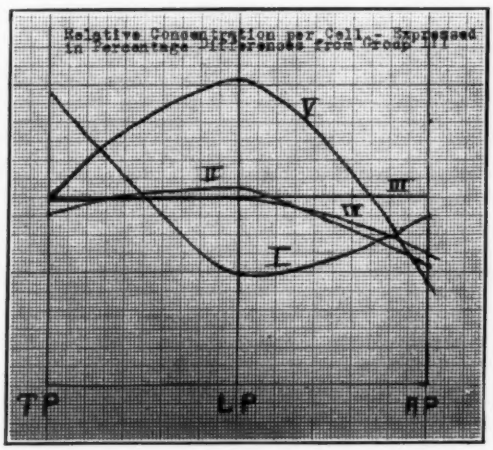


Chart V.

phosphorus fraction is confined to the cell. Within experimental error the sum of the lecithin and acid soluble phosphosus is equal to the total phosphorus in normal individuals and this is also true in Groups II, III, IV and V. The significance of this phosphorus residue clearly indicates that it is a fraction precipitated by the ether-alcohol mixture and the trichloracetic acid. Found coincident with the terminal stage of the disease is indicative that it may represent a nucleoprotein or an abnormal decomposition product of the latter or possibly a product of metabolism of the tubercle bacillus found either free or bound to some phosphorus compound in the cell.

From Table IV it is to be observed that the actual cell volume is decreased in all the groups, particularly Group I. A possible explanation is that the water is drawn out of the cell by either an increase in the osmotic pressure of the plasma occasioned by an increase in plasma solids or a disturbance in the water balance equilibrium between the blood and the tissues.

The phosphorus quotient is obtained by multiplying the ratio between the total phosphorus in 100 c.c. of cells and the number of red cells in the same

volume by $10^{10}$. In the same column, in parenthesis, are found the percentage variations from Group III. The phosphorus quotients of Groups III, IV, and V are identical while that of Group II is very slightly increased and Group I much more so.

It is not assumed that the white cells are phosphorus free, but the leucocytes are relatively few in comparison to the number of erythrocytes.

The inorganic phosphorus in the plasma is higher than previous reports on normal subjects. It apears to be practically equal to the acid soluble phosphoric acid fraction of the plasma. Denis[4] has recently reported that there is no inorganic phosphoric acid in the cell in the normal subject, while Bloor[5] previously reports an average of 18.8 mg. per 100 c.c. of cells. Since it was not possible to use the technic suggested by Denis in this study, not much emphasis can be placed upon the values obtained for inorganic phosphate in whole blood or cells; still it may represent a comparable fraction which if not present as inorganic phosphate is one that may readily be converted into the same.

Table VI shows a contrast between Group III and the average figures for normal men given by Bloor.[6] The most notable difference lies in the decrease of lecithin in the cell with an increase in the acid soluble phosphorus fraction in both the cell and the plasma. Even in the early stages of the disease there appears a tendency toward a decrease in lecithin and an increase in acid soluble phosphorus.

Charts VI and VII indicate the manner in which the lecithin and the acid soluble phosphates fluctuate from normal as the disease passes through Groups III to II to I, and III to II to V respectively. These percentage differences

TABLE IV

MG. OF $H_3PO_4$ PER INDIVIDUAL CELL

| GROUP | TOTAL PHOSPHORUS | LECITHIN PHOSPHORUS | ACID SOLUBLE PHOSPHORUS | PERCENTAGE VARIATION OF CELL VOLUME FROM GROUP III | PERCENTAGE VARIATION OF CORPUSCLE RATIO FROM GROUP III | PROSPHORUS QUOTIENT |
|---|---|---|---|---|---|---|
| I | $2.38 \times 10^{-10}$ | $.446 \times 10^{-10}$ | $1.74 \times 10^{-10}$ | −13.1% | −15.6 | 2.38 (+8.6%) |
| II | $2.15 \times 10^{-10}$ | $.480 \times 10^{-10}$ | $1.67 \times 10^{-10}$ | − 8.7% | + 0.7 | 2.15 (−1.8%) |
| III | $2.19 \times 10^{-10}$ | $.477 \times 10^{-10}$ | $1.77 \times 10^{-10}$ | | | 2.19 |
| IV | $2.18 \times 10^{-10}$ | $.476 \times 10^{-10}$ | $1.68 \times 10^{-10}$ | − 3.4% | − 1.0 | 2.18 (−0.4%) |
| V | $2.19 \times 10^{-10}$ | $.524 \times 10^{-10}$ | $1.64 \times 10^{-10}$ | − 5.4% | − 0.5 | 2.19 ( 0.0%) |

TABLE V

SHOWING PERCENTAGE CHANGES FROM GROUP III IN THE INDIVIDUAL CELL

| GROUP | TOTAL PHOSPHORUS | LECITHIN PHOSPHORUS | ACID SOLUBLE PHOSPHORUS |
|---|---|---|---|
| I | +8.6% | −6.5% | −1.7% |
| II | −1.8% | +0.6% | −5.6% |
| IV | −0.4% | −0.2% | −5.0% |
| V | 0.0% | +9.8% | −7.3% |

are obtained by comparison of the results in Tables V and IV (plasma) with the corresponding phosphorus content of the cell and unit volume of plasma of normal men calculated upon the basis of Bloor's figures assuming that the average volume of the normal male red cell is $90 \times 10^{-10}$ c.c. and that the number of erythrocytes per 100 c.c. is $111 \times 10^{8}$ cells. From Charts VI and

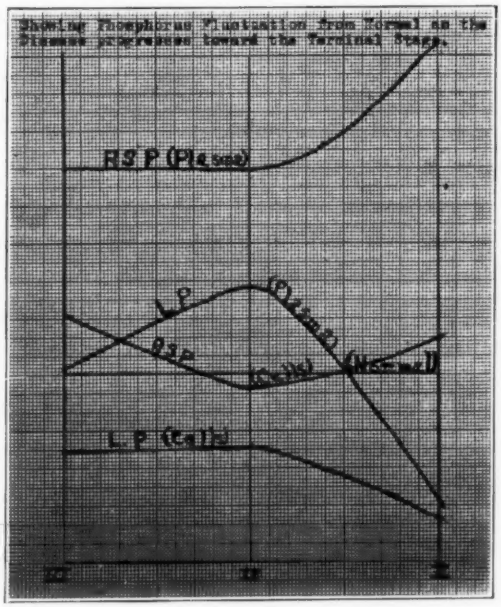
Showing Phosphorus Fluctuation from Normal as the Disease progresses toward the Terminal Stage.
R S P (Plasma)
L P
R S P
L P (Cells)

Chart VI.

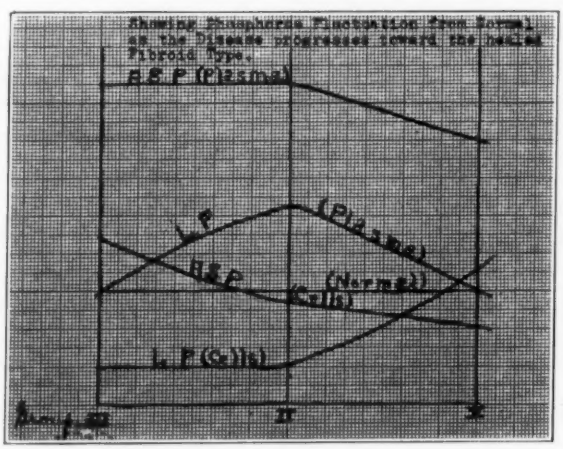
Showing Phosphorus Fluctuation from Normal as the Disease progresses toward the Terminal Fibroid Type.
R S P (Plasma)
L P
R S P
L P (Cells)

Chart VII.

VII, it is to be noted that in general the greatest variation occurs in the acid soluble phosphorus of the plasma and the lecithin content of the cell. As one fraction increases, the other simultaneously decreases and vice versa. There is a similar relationship existing between the lecithin content of the plasma and the acid soluble phosphorus of the cell, but these differences are not so exaggerated.

TABLE VI

MG. OF $H_3PO_4$ PER 100 C.C.

| GROUP | CELLS | | | PLASMA | | |
|---|---|---|---|---|---|---|
| | TOTAL PHOSPHORUS | LECITHIN PHOSPHORUS | ACID SOLUBLE PHOSPHORUS | TOTAL PHOSPHORUS | LECITHIN PHOSPHORUS | ACID SOLUBLE PHOSPHORUS |
| Bloor's Group Average | 248.0 | 57.0 | 188.0 | 32.0 | 22.1 | 10.4 |
| Group III Average | 239.7 | 52.0 | 193.8 | 34.5 | 22.4 | 12.2 |

## SUMMARY

1. There is a disturbance in phosphorus metabolism in tuberculosis.

2. A decrease in lecithin content of both the cell and the plasma with a simultaneous increase in acid soluble phosphorus represents grave prognosis while the opposite indicates a favorable condition.

3. Since the decrease in lecithin is accompanied by an increase in acid soluble phosphorus it would appear that the latter fraction may be utilized for the synthesis of lecithin. In the advanced stages of tuberculosis there may be a destruction of lecithin or an inability to synthesize it, occasioned by a lack within the body of fatty acids, the choline residue, glycerol or perhaps specific enzymes.

4. The size of the red cell is decreased in tuberculosis, except in the first stages, and the volume per cent of cells is decidedly decreased in the far advanced exudative type.

5. From Table II it is interesting to note the average age of patients in the different groups. It is to be observed that the youngest patients are found in Group III, indicating what has long been known that tuberculosis is a disease of early adult life. That the period of healing is proportional to the amount of involvement is observed by comparison of the two proliferative groups. If the disease is not arrested it passes in a few years to the terminal stage.

6. The sum of the lecithin and acid soluble fractions is equal to the total phosphorus except in Group I, terminal stage, in which the total phosphorus is greater than the sum of these two fractions. This additional phosphorus fraction is confined to the cell and represents a group not soluble in ether-alcohol or trichloracetic acid.

The author is indebted to Dr. H. C. Sweany for the classification of the patients used in this study and for which a kind appreciation is offered.

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# A NOTE ON THE DESTRUCTION OF COMPLEMENT IN HUMAN SERUM BY HEATING AT 55° C. FOR FIFTEEN MINUTES*

By Gordon Ernest Davis,† M.S., Mobile, Ala.

IN spite of the work done by Kolmer and others on the destruction of complement in human serum by heating at 55° C. for fifteen minutes instead of from twenty to thirty minutes as formerly used, there is still an occasional echo of doubt as to the efficiency of the shorter period of time. To determine this point for myself, I tested the serum from 500 routine blood specimens as they came to the laboratory from seven counties over a period of nearly two months. The Wassermann tests are begun on Tuesday and Friday of each week. All bloods are placed in the ice box as received and the serum removed just before testing.

I felt that if after inactivation the complement remained sufficient to even weaken a small part of the regulation dose of cells, then a longer period should be used.

A two-tube series was set up sufficient for the sera of the day. In the back tubes I placed 0.1 c.c. of raw serum and in the front tubes the same amount of inactivated (fifteen minutes at 55° C.) serum. One-tenth c.c. (one-fifth of the regulation dose) of the red cell suspension made up for the day was then added to all tubes and the total made up to 3 c.c. with physiologic saline. The back tubes thus acted as controls on the front tubes. All tubes were incubated at 38° C. for one hour.

Of the 500 sera, twenty-six failed to hemolyze before heating, i.e., these sera contained insufficient complement or native anti-sheep hemolysin, or both to produce hemolysis in the suspension of cells used. Of the 474 sera which gave complete hemolysis before inactivation, not one showed any detectable degree of hemolysis after inactivation. Five hundred sera is not a very long series but I feel that the results are fairly conclusive of the efficiency of the short period of inactivation.

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†Director, Mobile Branch Laboratory, Mobile, Ala.

# FIBROID GUMMA*

## (Juxta-Articular Nodules of Lutz-Jeanselme)

By Sigmund S. Greenbaum, M.D., Philadelphia

THE syphilitic gumma in the form of moderately firm asymmetrical nodules, running a characteristic course, as a rule towards ulceration in a fairly definite period of time, is well known; but that syphilis may be the primary etiologic factor in the production of very hard, often symmetrically located, intracutaneous and subcutaneous fibrotic nodules, almost pathognomonic when their clinical picture presents a certain topography, is practically unknown. Although the appearance of these nodules generally causes no symptomatic disturbances, they are important to recognize from a diagnostic point of view. There are few instances of this manifestation of syphilis, recorded as such, in the literature, nor have I been able to find mention of the condition in most of the current textbooks on syphilis.

Because of the recent observation of a typical example in a syphilitic, who had a primary lesion eleven years previously and who presented symmetrically on the elbows, on the right knee and on the left palm, very hard hypodermic and dermo-hypodermic nodules of six to eight years duration and because of their apparent rarity and the lack of uniformity regarding their etiologic significance, a clinical review of this combined syphilitic fibrogummatous lesion and its relation to the fibromata and the juxta-articular nodules, so common in the orient and tropical countries, appears desirable. The history of the patient forming the basis for this is as follows:

L. A., white male, salesman, aged forty-five, was referred by Alfred Cowan, March 13, 1924, with the diagnosis of chronic syphilis, a complement-fixation test having been performed because of recurrent attacks of corneal ulcer.

The patient was American born and had been married eighteen years. There was one child aged fifteen years; his wife had had one miscarriage preceding the birth of this boy and had one miscarriage after his birth but before the husband had contracted syphilis. The patient stated that he did not recall having any serious illness during his life except for the chancre which he had long since forgotten. The fibroid gummata caused him no trouble and were of no interest to him whatever. This is possibly the reason that so few instances of this condition have been noted in the literature. The first lesion to appear was on the hypothenar eminence of the left hand in the form of a hard nodule—the patient stated that it felt like a small marble right in the skin of the palm. The others appeared in the following order: left elbow, right elbow, thenar eminence left hand and lastly on the right knee. They had, at no time, been painful or tender. Those over the large joints had been soft and "spongy" at first, readily movable under the skin and had later become very firm. There was no history of a secondary luetic eruption and the patient stated that he had never felt better in his life.

*Examination.*—The patient, an exceedingly well-preserved and well-nourished man of medium height, weighed 162 pounds and was apparently in the best of health and spirits.

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Except for a moderately marked granular pharyngitis, the nose and throat examination was negative. The heart was apparently normal except for a slightly accentuated aortic second sound. The lungs and abdominal viscera were apparently normal. The systolic blood pressure was 130. The urinalysis was negative.

On both forearms, over the olecranon processes, about one to two inches above the elbow tips, were very hard nontender subcutaneous globular nodules. Although very freely movable, they were attached to the overlying skin to a definite degree by their superior poles. Although symmetrical as to position, they were not of the same size. The lesion on the left elbow was much larger and about the size of a moderate sized hen's egg. Of all the lesions, this was the most easily movable under the skin and was distinctly globular. The lesion on the left elbow was about the size of a pigeon's egg and had a very small hard additional nodule attached to its deeper surface laterally. The nodule on the right knee had the same characteristics as those on the elbows except that it was much smaller. The

Fig. 1.—Fibroid gummata over olecranon processes. (Juxta-articular nodules of Lutz-Jeanselme.)

lesions on the palms were somewhat different from the others. They were at the same time both intra- and hypodermic. They were about the size of navy beans and the skin over them was hyperkeratotic. None of the nodules bore any relation to the bursae.

The Wassermann test was four plus with all antigens and with several technics. No histologic examination was made as the patient refused to permit a biopsy.

*Treatment and Results.*—Within one week after the first injection intravenously of a small dose of neoarsphenamine (.30 gm.), the patient noted a perceptible diminution in size of the smaller lesions on the palms. By the fourth injection, there was distinct shrinkage in the size of all the lesions while the palmar lesions had lost all sensation of being nodular.

All that remained of the palmar lesions were hyperkeratotic spots. At the end of the first series of arsenical injections (12.6 gm.) the palmar lesions had completely disappeared and the juxta-articular nodules had regressed fully 75 per cent. There was almost complete disappearance of all lesions at the end of the second series of arsenical injections which had been preceded by 30 intramuscular injections of bismuth.

This case is a typical example of a condition observed by Lutz in 1891 and later by Jeanselme whose clinical description is classical and who gave it the term juxta-articular nodules.[1] At that time, Jeanselme stated that the condition was neither syphilitic nor tuberculous and a practical application of the Bordet-Gengou phenomenon had not as yet been discovered. More recently, in England and America, several cases, in which the clinical picture was similar to that of juxta-articular nodules, have been described by Weber,[2] Goodman and Young,[3] Goodman[4, 5] and Fox,[6] under the term subcutaneous fibroid syphilomata. That the two conditions are the same is admitted by Jeanselme in a recent review of the subject appearing during the preparation of this report,[7] although he does not as yet admit a common primary etiologic factor.

Juxta-articular nodules, as observed by Jeanselme among the Indo-Chinese, are rounded or poly-lobulated, hard, indolent, lesions of varied size, which, as their name indicates, tend to develop or specially locate near joints. In this special location, they tend to assume a remarkable symmetry and on this, chiefly, rests their diagnostic value. The nodules are at first subcutaneous and freely movable but later, often become attached to the overlying skin. After reaching a certain point in their development, they generally persist as such, although very exceptional instances have been recorded of softening, evacuation, ulceration and cicatrization. Since Jeanselme's original observation of this condition, it has been noted over widely distributed areas in the orient, Africa and south America, with apparently sporadic cases here and there in North America, England and the Continent. The majority of observers have been able to definitely ascertain that the common carriers of juxta-articular nodules are individuals with an antecedent history of syphilis or yaws and that the complement-fixation test for syphilis is frequently positive. In one instance, spirochetes were found in silver-nitrate impregnated sections of a nodule.[8] In the hands of most investigators, excellent therapeutic results have followed antisyphilitic, particularly arsenobenzene, therapy. It appears therefore that sufficient evidence has accumulated to definitely establish at least one of the factors in the production of juxta-articular nodules.

Connected with the etiology of juxta-articular nodules is the question of the etiology of fibromata in general. It is well known that the history of fibromata (the term in many textbooks includes the so-called true and pseudo-fibromata) is not always uniform and that the histologic appearance frequently depends upon the time, as related to the stage of development, when the section is made. A survey of the pathologic descriptions of fibromata shows that there is, at times, a fairly marked similarity between the description of some of them and the description of the histologic appearance of juxta-

articular nodules. As a matter of fact, the clinical appearance is that of multiple fibromata but it must be remembered that this is not the fact pathologically. They are not true fibromata. Nevertheless, it is of interest to note that Stelwagen, quoting Hashimoto, states that fibromata (?) are extremely common in the eastern countries whereas they are comparatively uncommon in this country and in Europe. Juxta-articular nodules usually occur at pressure points (ankles, knees, elbows). The positions assumed by the peoples in the eastern countries, when praying, resting, etc., as well as the tendency of the dark-skinned and the exceptional white-skinned individual to fibrotic hypertrophies explains the localizations and the pathologic composition of fibroid gummata. Since juxta-articular nodules are of extreme frequence in the areas similarly noted by Hashimoto for fibromata, the inference that many of these latter are really juxta-articular nodules or fibroid gummata is justifiable. They are really another illustration of the effect of trauma on the development of syphilitic lesions plus the fibrotic tendency of the individual.

There is a growing desire among dermatologists to avoid the term tubercle in describing a certain elementary skin lesion, and to substitute for it, wherever possible, the more descriptive term lupoma, leproma, etc. Just as the term lupoma or leproma replaces the obsolete term lupus tubercle, leprous tubercle, the term syphiloma should be restricted to the nonresolutive syphilitic tubercle in which there is dermic destruction with scar formation (cutaneous fibrosis) as the end-result.

The term fibroid gumma clearly describes the condition.

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# URIC ACID LEVELS IN THE BLOOD OF MAN AND ANIMALS*

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## INTRODUCTION

THE value of a reliable uric acid method for the control of such a disease as nephritis is obvious, but until quite recently no such methods were available. Formerly from 1 to 3 milligrams per 100 c.c. was considered normal. but recent work has shown that the methods used to establish this record were faulty. The purpose of this work therefore is to ascertain if possible the most reliable and at the same time the most practical method, to discover any source of error hitherto unrecognized, and to establish various levels of concentration for animals and man.

## URIC ACID METHODS

Prior to the introduction of the phosphotungstic acid reagent of Folin and Denis, uric acid was determined gravimetrically, a procedure which required 50 to 100 c.c. of blood, making analysis almost impossible for routine procedure. In 1913 Folin and Denis developed a colorimetric method by means of which it was possible to make an accurate determination on 20 c.c. of blood. In 1915, Benedict and Hitchcock modified the Folin and Denis method so that it was possible to make an analysis on 10 c.c. of blood. In 1919, Folin and Wu first described the use of tungstic acid wherein they were able to obtain a protein free filtrate on which all nonprotein constituents of the blood could be determined. They were able to make the uric acid analysis on sufficient of this filtrate, to represent 2 c.c. of blood. In 1922, Benedict[1] introduced a new arsenic-tungstic acid reagent with which he brought out a very accurate method for uric acid in the Folin-Wu[2] tungstic acid filtrate. In Benedict's new technic, the test was made on this filtrate representing 0.5 c.c. of blood.

The Folin-Wu method, using the equivalent of 2 c.c. of blood was epoch-making. The determination was made by precipitating the uric acid from 20 c.c. of Folin-Wu filtrate as the silver urate by means of silver lactate-lactic acid solution. The urate was thrown down in a centrifuge, separated from the supernatant liquid by decantation and then dissolved by a hydrochloric acid solution of sodium chloride. The chloride solution of the silver urate was transferred to a 25 c.c. graduated flask, made alkaline with sodium carbonate and sodium cyanide. A uric acid standard was prepared containing equal amounts of carbonate and cyanide as used in the blood analysis and a blue color developed by adding Folin and Denis reagent.[7] This method has been criticized by Rogers,[6] Benedict[1] and by Brown and Raiziss.[4]

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H. Rogers has shown that the Folin-Wu method is subjected to a very serious source of error when silver urate is exposed to light. We also noted that this darkening of the precipitate gave low results and had always prevented it. Benedict points to the facts: that in the Folin-Wu method, the uric acid is precipitated from a blood filtrate where its actual concentration may be even less than one part in a million; that uric acid cannot be precipitated in pure solution of ten times the concentration existing in blood filtrates; that the color produced is too weak to make accurate comparisons with standards; that the sulphite standard was subject to slow deterioration; and that sulphites interfered with the development of blue color in the analysis. Brown and Raiziss state that the results from the Folin-Wu test are 6 to 10 per cent too low but those from the new Benedict technic are too high. We have not been able to agree with the authors on these points or to the effect of polyphenols and aminoacids on the color development in uric acid analysis with the Benedict or Folin and Denis reagent (See Tables IV and V).

In Benedict's new method, the test is made directly on 5 c.c. of Folin-Wu filtrate in a weak sodium cyanide solution. The color is developed by an arsenic-tungstic acid reagent, by heating three minutes in a boiling water-bath. The color so produced is about three times as strong as that developed in the Folin-Wu method and better colorimetric readings can be made thereby. In Table I, are shown the results of analyses using the new Benedict and the technic of Folin-Wu. In seventeen of twenty cases the Benedict gives higher results than the Folin-Wu. Following this in Table I, are shown the results of the new Benedict and Benedict's modification[1] of the Folin-Wu, uric acid method. In seventeen of eighteen analyses the Benedict technic gives higher results.

In Table II, results are given by the new Folin[3] and the new Benedict[1] methods, each method giving a fairly accurate per cent of recovery. Folin's supplement to "A System of Blood Analyses"[3] deals with the criticism of Pucher[10] and Benedict,[1] and outlines a new direct colorimetric method for uric acid similar to that of Benedict, using 5 c.c. of the Folin-Wu filtrate with the Folin and Denis reagent. This results in the development of a much stronger color than in any of the previous methods. Furthermore, by employing lithium salts instead of sodium, Folin has eliminated any chance of cloudy solutions that may arise from time to time in making analysis as outlined by Benedict.

## EXPERIMENTAL

Comparisons between the new Folin and new Benedict technics were then carried out and at the same time a method, using the Folin technic with a mixed reagent of three parts of Folin and Denis to one part of the Benedict arsenic-tungstic acid reagent. The results of twenty-seven analyses are shown in Table III. The difference of the two methods is always very small and within the limits of experimental error, a fact not true with the old Folin-Wu when compared with Benedict's method. As the Benedict arsenic reagent is supposed to give an increase and the Folin and Denis reagent a decrease in

color in the presence of polyphenols, by adding one part of the former to three parts of the latter, a reagent was made that should not give an increase or decrease in color with interfering substances. However, this mixed reagent gave the same results as the other two with bloods of normal phenol and uric acid content, as shown in Table III.

Analyses were then made to determine the excess color development with reagents on the addition of polyphenols to the blood. Phloroglucin (1-3-5- trihydroxyphenol) was added in the ratio of 3 mg. to 100 c.c. of blood giving double the concentration of phenols found in normal bloods. The results of Table IV show that the effect of polyphenols on the uric acid determination is negligible when using either the Benedict or Folin and Denis reagent.

In Table V are results obtained by adding equal parts of tyrosine and aspartic acid equivalent to 7 mg. of amino-acids per 100 c.c. of blood. Even

TABLE I

| NUMBER BLOODS TESTED | DIAGNOSIS | AVERAGE S. R. BENEDICT'S TECHNIC | AVERAGE FOLIN-WU TECHNIC | DIFFERENCE BENEDICT'S AND FOLIN'S |
|---|---|---|---|---|
| 2 | Incipient TB. | 4.77 | 3.86 | 0.91 |
| 10 | Mod. advanced | 4.14 | 3.06 | 1.02 |
| 4 | Far advanced | 3.14 | 3.13 | 0.01 |
| | | S. R. Benedict's technic | Benedict's Modification of Folin-Wu Technic | |
| 1 | Incipient | 3.7 | 3.0 | 0.7 |
| 6 | Mod. advanced | 3.82 | 2.88 | 0.94 |
| 6 | Far advanced | 3.63 | 2.68 | 0.95 |
| 2 | Nephritic | 5.4 | 5.4 | 0.0 |
| 1 | Uremic | 4.7 | 5.8 | −1.1 |
| 4 | Normal | 4.06 | 3.06 | 1.0 |

NOTE: Results in mg. per 100 c.c. of blood.

TABLE II

RECOVERY OF URIC ACID ADDED TO THE BLOOD BY BENEDICT'S NEW AND FOLIN'S NEW METHODS

| CASE NO. | TECHNIC USED | URIC ACID IN BLOOD | URIC ACID ADDED MG. PER 100 C.C. | TOTAL URIC ACID RECOVERED | ADDED URIC ACID RECOVERED | PER CENT RECOVERED |
|---|---|---|---|---|---|---|
| 16969 | Benedict | 3.3 | 3.0 | 6.3 | 3.0 | 100.00 |
| | Folin | 3.3 | 3.0 | 5.9 | 2.9 | 96.66 |
| 16982 | Benedict | 3.45 | 2.0 | 5.5 | 2.05 | 102.5 |
| | Folin | 3.50 | 2.0 | 5.5 | 2.00 | 100.0 |
| 16947 | Benedict | 3.64 | 2.0 | 5.60 | 1.96 | 98.0 |
| | Folin | 3.20 | 2.0 | 5.65 | 1.95 | 97.5 |
| 16882 | Benedict | 3.44 | 2.0 | 5.35 | 1.91 | 95.5 |
| | Folin | 3.50 | 2.0 | 5.60 | 2.10 | 105.0 |
| 16910 | Benedict | 3.30 | 2.0 | 5.4 | 2.1 | 105.0 |
| | Folin | 3.40 | 2.0 | 5.6 | 2.2 | 110.0 |
| 16951 | Benedict | 2.3 | 2.0 | 4.25 | 1.95 | 97.5 |
| | Folin | 2.6 | 2.0 | 4.70 | 2.10 | 105.0 |
| Guinea Pig (1) | Benedict | 2.7 | 2.0 | 4.8 | 2.1 | 105.0 |
| | Folin | 2.5 | 2.0 | 4.7 | 2.2 | 110.0 |
| Rabbit (10) | Benedict | 2.8 | 2.0 | 4.2 | 1.4 | 70.0 |
| | Folin | 2.9 | 2.0 | 4.9 | 2.0 | 100.0 |
| Horse (4) | Benedict | 3.4 | 2.0 | 5.4 | 2.0 | 100.0 |
| | Folin | 3.4 | 2.0 | 5.4 | 2.0 | 100.0 |
| TB. dog | Benedict | 1.3 | 2.0 | 3.3 | 2.0 | 100.0 |
| L. O. B. | Folin | 1.6 | 2.0 | 3.3 | 2.0 | 100.0 |

TABLE III

THE RELATION OF URIC ACID TO THE OTHER NITROGEN CONSTITUENTS OF THE BLOOD: THE COMPARISON OF BENEDICT'S NEW AND FOLIN'S NEW COLORIMETRIC TESTS: The Results on a Mixed Benedict—Folin Denis Reagent.*

| NO. BLOODS TESTED | DIAGNOSIS | TOTAL NITROGEN | NON-PROTEIN NITROGEN | UREA NITROGEN | AMINO-ACID NITROGEN | PRE-FORMED CREATI-NINE | TOTAL CREATI-NINE | REST N. | URIC ACID BENEDICT | URIC ACID FOLIN | DIFFER-ENCE BENEDICT-FOLIN'S | *BENE-DICT AND FOLIN | DIFFER-ENCE FROM BENEDICT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 8 | Incipient | 2835.0 | 27.45 | 12.25 | 7.12 | 1.38 | 4.74 | 7.25 | 3.92 | 3.82 | 0.10 | 3.86 | 0.06 |
| 5 | Mod. Advanced | 3021.8 | 24.58 | 11.70 | 6.71 | 1.28 | 4.40 | 6.15 | 3.37 | 3.30 | 0.07 | 3.34 | 0.03 |
| 5 | Far Advanced | 2954.0 | 26.46 | 11.3 | 6.85 | 1.38 | 4.79 | 6.76 | 3.27 | 3.29 | 0.02 | 3.47 | 0.20 |
| 3 | Normal | 3366.6 | 32.9 | 13.03 | 7.3 | 1.40 | 4 9 | 10.9 | 3.60 | 3.53 | 0 07 | 3.55 | 0.05 |
| 3 | Nephritis | 1740.0 | 37.96 | 17.46 | 7.40 | 1.70 | 5.2 | 11.3 | 3.63 | 3.62 | 0.01 | 3.67 | 0.04 |
| 1 | Gastric Carcnioma | 3300.0 | 27.8 | 12.9 | 5.6 | 0.80 | 3.34 | 7.5 | 4.0 | | | | |
| 1 | Rheumatic Fever | 1330.0 | 44.0 | 28.1 | 8.8 | 1.40 | 5 0 | 0.0 | 2.1 | 2.1 | 0.0 | 1.95 | 0.15 |
| 1 | Acute Articular Rheumatism | 2120.0 | 38.8 | 15.5 | 8.3 | 1.50 | 4.8 | 13.3 | 3.6 | 3.2 | 0.4 | 2.96 | 0.64 |
| 1 | Asthma | 3460.0 | 33.4 | 16.7 | 7.0 | 1.10 | 5.0 | 8.0 | 4.0 | 3.7 | 0.3 | 4.0 | 0.0 |

*The New Folin technic was used with a reagent containing three parts of the Folin and Dennis and one part of Benedict's arsenic tungstic acid reagent.

The results given in the table are the average of the number of bloods given in Column I.

TABLE IV

RESULTS OBTAINED BY ADDING (1-3-5) TRIHYDROXYPHENOL (PHLOROGLUCIN) (EQUAL TO 3 MG. PER 100 C.C.) TO BLOODS IN URIC ACID DETERMINATIONS

| INITIALS AND CASE NO. | TECHNIC USED | URIC ACID IN BLOOD | APPARENT URIC ACID CONTENT | COLORIMETRIC INCREASE CAUSED BY ADDING PHLOROGLUCIN | PER CENT INCREASE |
|---|---|---|---|---|---|
| I. G. 17018 | Benedict New | 2.67 | 2.86 | 0.09 | 0.371 |
| | Folin New | 2.60 | 2.70 | 0.10 | 0.385 |
| C. T. 17017 | Benedict | 3.69 | 3.97 | 0.28 | 0.759 |
| | Folin | 3.40 | 3.55 | 0.15 | 0.441 |
| N. Gears 17016 | Benedict | 2.76 | 2.96 | 0.20 | 0.724 |
| | Folin | 2.70 | 2.93 | 0.23 | 0.851 |

TABLE V

RESULTS OBTAINED BY ADDING EQUAL PARTS OF TYROSINE AND ASPARTIC ACID (EQUAL TO 7 MG. PER 100 C.C.) TO BLOODS IN URIC ACID DETERMINATIONS

| INITIAL AND CASE NO. | TECHNIC USED | URIC ACID IN BLOOD | APPARENT URIC ACID CONTENT | COLORIMETRIC INCREASE CAUSED BY ADDING AMINOACIDS | PER CENT INCREASE |
|---|---|---|---|---|---|
| C. T. 17017 | Benedict New | 3.69 | 3.65 | −0.04 | −0.17 |
| | Folin New | 3.40 | 3.40 | 0.00 | 0.00 |
| M. Gears 17016 | Benedict New | 2.76 | 2.66 | −0.10 | −0.36 |
| | Folin New | 2.70 | 2.80 | 0.10 | 0.37 |
| I. G. 17018 | Benedict New | 2.67 | 2.30 | 0.37 | 1.38 |
| | Folin New | 2.60 | 2.60 | 0.00 | 0.00 |

with double the amounts of amino-acids present as found in normal bloods, only a slight increase in color was obtained by either reagent.

From the results of Table II, the fact is shown that most animals have a small amount of uric acid in the blood as determined by the newer methods of Benedict or Folin, although Brown and Raiziss[4] found the opposite results by the old Folin-Wu technic. As Benedict has shown that the Dalmatian dog was the only animal besides man and the higher apes to excrete uric acid, it is evident from these results that there must be disintegration of uric acid before excretion. More recently Folin has shown this destruction to take place in the kidney cells and to vary in different individuals.

It has been demonstrated above, that very accurate determinations of uric acid can be made on one-half to one c.c. of blood, so that at the present time, work may be done on exogenous and endogenous uric acid metabolism in any phase of any disease.

From the results given on human blood in Tables I, II, and III it would seem that the question raised by Benedict as to the threshold for normal blood might be answered. The total and nonprotein nitrogen constituents of the bloods in Table III are normal in many cases while the uric acid content is from 3 to 4 mg. per 100 c.c. of blood. Therefore, it is evident that this "normal level" should be raised from 1 to 3 mg. to 1 to 4 mg. of uric acid per 100 c.c. of blood.

Tables I and III show the uric acid content of bloods in tuberculosis. It must be remembered that the plasma has about twice as much uric acid

concentration as the corpuscles and that plasma is increased in advanced tuberculosis from 55 per cent to 70 per cent of the whole blood. However, most tests give results of uric acid content about normal in all phases of this disease, with a tendency to decrease as the disease becomes far advanced. This therefore means a slight actual decrease as well as a slight relative decrease in advanced tuberculosis.

## SUMMARY AND CONCLUSION

1. The Folin-Wu test does not give results equal to the total amount of uric acid in the blood.

2. Folin's and Benedict's latest methods give the same results and each give a fairly accurate recovery of uric acid added to human or animal bloods.

3. Polyphenols and amino-acids in quantities double those found in normal bloods do not make appreciable error in uric acid determinations by the Folin or Benedict methods.

4. The normal level for uric acid in the blood should be raised from 1 to 3 mg. to 1 to 4 mg. per 100 c.c. of blood.

5. Uric acid content of bloods in all stages of tuberculosis is about normal with a very slight actual as well as relative decrease in the far advanced stages.

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# BETTER POSTMORTEM SERVICE*

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TALKING about postmortem service to a group like this is a good deal like bringing coals to Newcastle. You are, all of you, primarily or secondarily interested in good postmortem service. In fact, for some of you it is perhaps one of the main objects of work, although it is often not considered directly applicable to the work of a clinical pathologist. However, it is a subject in which every one of us finds certain phases of interest at times, and these phases I want to discuss with regard to the points that appear to be especially worth while.

One of the fundamental factors with regard to death is the obligation which *must* be assumed by any physician or group of physicians under whose care a patient dies. The contractual relation which exists between a patient and his physician is recognized far and wide, both legally and morally. The physician agrees to give his best service; the patient agrees to submit himself to the physician, and afterward to pay a reasonable fee. This contractual relation is so prominent that sometimes we are inclined to think it is the only one. There is another relation, however, that is more fundamental, and which is not so easily recognized, and that is the obligation that is brought on every physician or every group of physicians by the death of a patient under their care. When once that obligation is sensed, the most fundamental factor that has to do with the progress of the practice of medicine is sensed. When a patient dies, a great debt is owed to humanity in order that the patient shall not have died in vain, and it is our duty as practicing physicians (not necessarily as pathologists) to see that the utmost possible benefit to our future patients, to the physician, and to humanity as a whole, shall come from this death. Why do we not all assume that obligation in a greater degree? Why do we not all obtain permission for examination, and review the deaths in 100 per cent of our cases? More simple still, why do we not all *ask* for permission in 100 per cent of cases? A number of reasons will readily occur to all of you, but I shall mention one of the most potent which may not have occurred to all of you. It has to do with the recognition of the very obligation I refer to. It has to do with the fact that the physician himself has not had "sold" to him the idea of the necessity and value of postmortem examinations. You will say that is certainly not true, for what physician would say that the postmortem examination is not valuable? Yes, the postmortem examination on *somebody else*. The other day a medical student from one of our leading medical colleges, in his last year in the medical school, was approached on the subject of postmortem

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examination on his father. He said, "I hope you will not insult me by asking permission for an examination in the case of my father." This is not a rare instance. Such "insult" would be inferred by an alarming percentage of physicians throughout the country and just so long as this attitude maintains, we shall have a barrier of inertia against the obtaining, and against the enthusiasm for, postmortem examinations in our hospitals. We do not need to bring it back to the usual pleas that "people don't want examinations," and "we must not offend them by asking for it," and "these people are so peculiar they don't like to have examinations." Let us bring it where it belongs, in many instances, and that is right up against the attitude of the physician himself. You can test it out. Test it out in your own mind. Test it out in the minds of your fellows. Are you going to permit a postmortem examination in the event of the death of one of your family? If you are not, I tell you now you have no right to ask anybody for permission to perform an examination on the bodies of their loved ones. I feel very strongly about this, because I have lived for a good many years (fifteen) under the mistaken and somewhat youthful enthusiasm that the doctor's interest and personal responsibility for the postmortem examination was a thing to be taken for granted, and I found it an extremely rude surprise when I began to investigate the subject closely, to ascertain that this interest not only could not be taken for granted, but that far and wide in a subtle way there was a feeling among doctors that "this is all right for poor Yorik in the city hospital; it may be all right for the fellow who is picked up in the street, but for one of the members of my family, for my friends, this is an insult." I wish to emphasize that every physician who has a person die under his care is in honor bound, and under obligation to humanity, to ask permission for a most thorough painstaking examination, made with the utmost possible respect, and with the idea that that death is going to be of value to humanity because of the things revealed. When doctors say to us, "You get permission here at the Mayo Clinic in 85 or 90 per cent of your cases, of course, because you have here a different class," they raise the red flag to the bull about as quickly as in any way I know, because we do not see very much difference in the people coming here and the people coming to other physicians. One reason why we get permission is because we are willing to grant permission on our own relatives; that is, we have that fundamental attitude. I want to stress this very point, that you have got to "sell" the idea of postmortem examinations to the doctors themselves. Fundamentally, they are not convinced. Another reason for our success is because we approach the relatives with the idea that we think this is the thing to do, and we think this is the way in which we are going to be able to serve humanity a little better.

A few points about the practical performance of a postmortem should be emphasized. One of them is, if a postmortem is worth doing at all, it is worth doing well. You are committing, in spite of what I have said, a certain sacrilege on the bodies of dead persons by the performance of a postmortem; and it does not make any difference who they are. It is obligatory upon you, therefore, that everything you do should be done most thoroughly.

Consequently you should learn to perform a postmortem properly, and if you do not know how, find out; you can do this even by reading. Anybody can make a good postmortem examination. There is no excuse for doing a poor one. If almost any doctor can be made a surgeon (and with all due respect to the surgeons, it would seem almost as if that were true), then, certainly almost any pathologist can learn to do a postmortem; and yet a postmortem well done, not necessarily expertly but carefully and systematically, should be much more common than it is. Another thing: take the relatives into your confidence; explain to them just why you want to make the examination, and after you finish, show them what you found. They will be tremendously interested. Make it of value to them; tell them what the chances are, so far as their inheritance is concerned, and with regard to vascular disease, discuss with them the pathogenesis that exists in the relative who has passed away; discuss with them the meaning of these tuberculous lesions in the chest; discuss the manner of living, what it may have meant to the deceased and may mean to the living relatives. Discuss the causes of death, so far as you can; lay your cards on the table; you will find that you will be met a good deal more than half way. You say, "I don't want to talk to this poor wife; she is all broken up." The one thing she wants to talk about is her husband's sickness, or her child's sickness; such discussion is the outlet for her emotion, and you are doing her a service. I know of nothing more comforting to relatives than to discuss frankly and freely the conditions that brought about the death of their loved ones. In the course of three years of discussions with relatives I have not seen one who did not feel better because he or she has had a chance to talk about these things openly. The screen that we draw between the patients and our practice should be torn down almost completely. If we do not tear it down, it will be torn down for us. People are getting more and more information concerning these subjects, and we must take this into consideration.

Another point: be sure to cooperate with the undertaker, who is in a position to render the most important opposition. Cooperation can be easily arranged. When I came to Minneapolis a number of years ago, I found that in order to make an examination one had to make it before the undertaker got there. If he could possibly prevent it, he would. Before I left the University of Minnesota at Minneapolis I had the unique experience of being called up by an undertaker and asked to make an examination because he felt the relatives should know the cause of death. In this particular case he had obtained permission for it, the physician in charge not having anything to do with it. Such a state of affairs is easily brought about. In the first place, remember that the undertaker, and the pathologist also, assume certain obligations toward the decent repair of the body; consequently, the pathologist should learn how to prepare a body after death. There is nothing complicated about this procedure although the undertaker's charges sometimes make one think differently. On the one hand certain measures on your part will aid the undertaker, and on the other hand, other measures may make it almost impossible for him to do a decent job. Again and again I have seen

undertakers frothing at the mouth with the condition in which the remains were left, and they were expected to bear the brunt of the difficulty, simply because the physician did not understand his task. In many instances I have called in the undertaker and explained to him that I wanted to do this or that, and asked his help. In other words, I have treated him as I would a cooperator in any other form of effort. Help him in his work and you will find very soon that he is willing to help you, because he wants to work with you instead of working against you.

Another important point is the preservation of gross specimens. Do not ask your staff to be interested or excited about a lot of dirty, foul-smelling, bloody stuff that you bring before them. The physician who is not accustomed to postmortem work does not like to get his hands or his clothes, or anything, dirty and covered with blood. This appears strange to some pathologists, but nevertheless it is true. The physician does not like to smell these specimens. This seems strange too, but it should be remembered that he was not brought up in such an atmosphere. There is no excuse for dirty, bad specimens. If this idea of selling postmortem examinations to the staff is to be developed, specimens must be preserved in a decent, presentable manner. Elaborate equipment is not necessary. Buy a large stone crock and put a cotton band around the lid, thus making it fairly tight, and in the stone crock float tissue attached to a cork by a string. The solutions, which may be prepared according to several formulas, we are now using in such a manner as not to require alcohol. Good specimens will be obtained which the surgeon will be glad, instead of sorry, to look at. One doctor says, "I have not had time yet to go into the question of gross specimens," and yet he is doing postmortems right along and showing the surgeon the result of his work. The only way to make postmortems popular is to show the surgeons specimens which they can appreciate, and which do not disgust them by their appearance. Remember that it is a simple matter to preserve specimens. Mounting in expensive glasses, and in an expensive solution is not necessary; they can be put in cheap jars with a piece of fiber label tied to them. Pieces of sections of every bit of tissue should be preserved for microscopic study. Some of the most amazing facts have been disclosed through the study of microscopic sections. One more point: insist that among your colleagues there is an open, free and frank discussion of this particular postmortem examination. It does not make any difference if you make only one examination every three months. Have a meeting of the staff on the poor fellow and go over his case in extenso. If there is anything about that case that the clinician did not understand, make him understand it. If there is anything that comes out of that case that will lead him or you to give better service to the next particular patient who has that disease, bring it out. If there is any way in which you can teach the surgeon to come out and face openly that he performed an appendectomy, and the patient died from peritonitis, and the chances were that the abdomen was infected at the time of operation, you have done a great work. Of course, you have to be a little careful how you put it. The surgeon may be a bigger man than you are;

nevertheless, if there is any way (I am overemphasizing this, of course) by which that postmortem can be made to teach the physician to face the issue openly, you have taken a tremendous step in advance. Clinicians and surgeons do not like to face the issue. Even pathologists do not like to admit they are wrong, and in this respect they are almost as human as the rest of the doctors who do not like to admit they are wrong; they prefer in some way or another to gloss over that thing we call "reputation." Keep it among your own crowd and do it quietly, and the next time a like case comes up and you have to face the issue, you will be a good deal more willing to say, "Yes, that man came to me. I wish I had not taken out his appendix; I knew he had a weak heart, but I didn't pay enough attention to an examination of his heart, but the next fellow that comes to me like that is going to have a good examination of his heart." When you can get results like that you are accomplishing something and your patient will not have died in vain.

*(For discussion, see page 505.)*

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## Boylston Medical Prizes of Harvard Medical School

The Boylston Medical Prizes, which are *open to public competition,* are offered for the best dissertation on questions in medical science proposed by the Boylston Medical Committee. The Committee is appointed by the President and Fellows of Harvard College. The names of the Committee appear in the annual catalogue of the Harvard Medical School.

A prize of five hundred dollars and the Boylston Prize Medal is offered every three years for the best dissertation on the results of original research in medicine, the subject to be chosen by the writer. The Boylston Prize Medal will be added to the money prize only in case the winning essay shows special originality in the investigations detailed. In awarding these prizes, preference will be given to dissertations which exhibit original work; if no dissertation is considered worthy of a prize, the award may be withheld.

Dissertations entered for this prize must be in the hands of the Secretary on or before December 31 of the year in which the prize is offered.

Each dissertation must bear, in place of the author's name, some sentence or device, and must be accompanied by a sealed packet, bearing the same sentence or device, and containing the author's name and residence within.

*Any clew by which the authorship of a dissertation is made known to the Committee will debar such dissertation from competition.* Previous publication of the work, if in form to give a clew to authorship, will debar from competition.

Dissertations must be printed or typewritten.

All unsuccessful dissertations are deposited with the Secretary, from whom they may be obtained, with the sealed packet unopened, *if called for within one year* after they have been received.

By an order adopted in 1826, the Secretary was directed to publish annually the following votes:—

1. That the Board does not consider itself as approving the doctrines contained in any of the dissertations to which premiums may be adjudged.
2. That, in case of publication of a successful dissertation, the author be considered as bound to print the above vote in connection therewith.

The address of the Secretary of the Boylston Medical Committee is Dr. Henry A. Christian, Peter Bent Brigham Hospital, Boston, Mass.

# *LABORATORY METHODS*

## MODIFICATIONS OF THE SAHLI HEMOGLOBINOMETER*

By Wm. C. Thro, M.D., New York City

SINCE the determination of the hemoglobin is one of the most important tests made on the blood and all laboratory workers have difficulties with different types of hemoglobinometers, it seemed that the following experiments might be of interest.

In spite of the fact that it is frequently stated that it would be better to express the amount of hemoglobin in grams per hundred, it is the universal custom to express it in percentage. It seems then that the standard of comparison may be a mixture of bloods of normal healthy human beings or a blood in which the hemoglobin is normal as determined by some chemical test like the oxygen combining power.

In working with classes of medical students for a number of years, it has been found that a more accurate comparison can be made with brown colors than with shades of red. The reds are used in hemoglobinometers like those of Fleischl, Fleischl-Miescher and Dare.

Our experience has been that the Dare instrument is easy to use and is the one to be preferred when fair accuracy is desired. It is rather an expensive instrument, however, and occasionally the two pieces of colored glass used as a standard are not well matched, and a number of students reading the same blood will get results varying from 5 to 10 per cent.

One of the chief faults of the Sahli is that the color standard fades, a conclusion arrived at by many laboratory workers. On opening a number of the standard color tubes (made by several manufacturers) and taking measurements of the bore, we were astonished to find variations from the required 7 mm., of from 7.3 to 6.6 mm. as measured by a Brown and Sharpe metric inside micrometer. These are the actual measurements of the bore of fourteen standard color tubes: 6.65, 6.71, 6.74, 6.81, 6.85, 6.86, 6.92, 6.93, 6.95, 6.99, 7.0, 7.03, 7.05, 7.35. However, when a local glass manufacturer made us some of these standard color tubes, it was found that the variations in the bore varied more than in the above. This only shows that the preparation of such apparatus, where accuracy is required, cannot be turned over to people who are not acquainted with the importance of a variation of a millimeter in the thickness of a colored solution.

Since we believe that, if properly made, the Sahli is one of the best hemoglobinometers, we proceeded with the idea that it could be made more accu-

*Received for publication, June 28, 1924.

rate if certain alterations were made. In the instrument at present on the market, the color tube and the graduated tube are 7 mm. apart, but a more accurate reading could be made if the two tubes were nearer together. It is therefore advisable to have the two tubes separated only by a blackened partition about 1 mm. thick. In using the Sahli, nearly everyone, when making the comparison, covers the upper portions of the tubes with the hand so that only a small length of both is visible. An instrument was therefore constructed in which the entire length of both tubes was hidden except a portion that is visible through an aperture 16 mm. × 16 mm. The accompanying figure is an outline of such a model. There is a small piece of ground-glass at the back. The base is of lead to make the instrument more stable.

The tube which is to contain the standard color is made like a small test tube with accurately gauged bore exactly 7 mm. in diameter and closed with a rubber stopper. It was admitted by one manufacturer, when he was shown

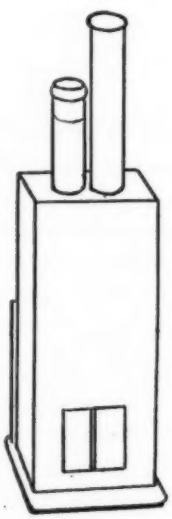

Fig. 1.

a very dark color tube, that careless heating of the glass tube to seal it causes a darkening of the solution. If the color changes in the stoppered tube, the material may be easily poured out and the tube cleaned and dried, sterilized and refilled with fresh 1 per cent acid hematin stock. We have found that if the comparison color mixture is made up and put into a brown glass bottle which is kept in the dark, that the color will not change in four years.

Make the standard hematin solution of:

| | |
|---|---|
| Blood oxalated | 1 c.c. |
| HCl tenth normal | 30 c.c. |
| Glycerine | 69 c.c. |

Sahli finally used a mixture of 1 per cent blood in water.*

Use the blood of an individual on whom the oxygen combining power has been determined or pool the blood of several healthy individuals.

*Sahli's Diagnostic Methods, Trans. by N. B. Potter, 1911, Edition 2, p. 749.

Sahli used as a comparison solution the acid hematin of the blood of individuals who evidently had a very high hemoglobin content, since with the instruments that came from Switzerland it was necessary to make a correction. For example, a reading of 65 equals 100 per cent.

For six years we have conducted the following experiment in the class in clinical pathology.

With the apparatus that comes with the Sahli hemometer, each student makes a 1 per cent acid hematin solution of his blood and this is poured into a flask. After all the solutions are added, the flask is well shaken and a portion of the mixture taken by each student and placed in the color tube and a hemoglobin determination made on his or her blood. (Before performing the experiment the student had some practice in the use of all the common hemoglobinometers.) These are the results:

| YEAR | STUDENTS | AVERAGE |
|---|---|---|
| 1918 | 50 | 94.0% |
| 1919 | 37 | 99.0% |
| 1920 | 40 | 100.0% |
| 1921 | 58 | 95.0% |
| 1922 | 53 | 98.8% |
| 1923 | 55 | 100.8% |

A variation of from 4 to 5 per cent is permissible in the reading and it is to be noted that the percentage comes very close to 100 per cent. With some very robust students, who had also a high red blood cell count, the percentage ran considerably above 100 and with others it was below 90, but it is interesting to note that the average in the six years was very close to 100.

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## A COLLECTING TUBE FOR SERUM*

By William G. Exton, M.D., New York City

IT is often necessary or desirable to delay the making of Wassermann and other tests of sera until some practicable or convenient time after collection of the specimen. It is also frequently impossible or impracticable to keep the specimens on ice during this delay. When the time that elapses between collection and testing is not prolonged, or when the sample of blood does not tend to hemolyze, the aseptic collection of blood by means of the vacuum ampule, introduced by Keidel for this purpose, is efficient and convenient. When, however, it is desired to provide for the satisfactory preservation of sera for days at a time and at room temperature in order to meet conditions incidental to getting specimens from remote localities to the laboratory through the mail, such as the conditions incidental to the Prudential Longevity Service, some other and more reliable method of preservation was found necessary.

Experiments showed that sera which were separated immediately from

*From the Prudential Longevity Service.
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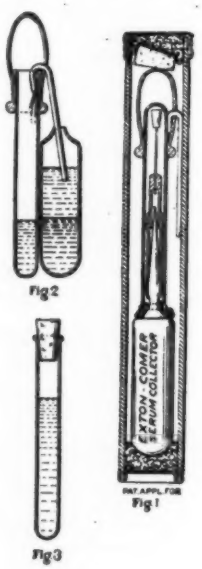
blood collected under aseptic conditions would not, of course, hemolyze, and also that a week or ten days, even a month or more, might elapse under mailing conditions without the sera becoming anticomplementary. After trying out a number of devices designed to accomplish the separation of serum conveniently, the one here illustrated was adopted and has proved very satisfactory in practice.

Fig. 1 shows the serum collector packed in a mailing case and ready for shipment. After removing the collector from the mailing case detach the small test tube (Fig. 3), which acts as a needle cover, and transfer the rubber nipple from the closed glass end slipping it over the mouth of the small test tube. Remove the stylus with the small attached cork from the needle point, and collect the blood in the usual way as with a Keidel tube. The tube with the blood may then be centrifuged (if a centrifuge is available) or left standing




in an upright position in a glass of ice water or at room temperature. When the clot forms and the serum separates, snap off the stem of the ampule at the small file scratch.

Fig. 2 shows the manner in which the separated serum is transferred from the collector to the small test tube. Press the rubber nipple so as to force the air out of the siphon tube, and, taking care not to disturb the blood clot, place the longer tip of the siphon tube near the bottom of the layer of clear serum. When pressure on the nipple is released the serum will be drawn rapidly into the small tube, which is stoppered with the cork provided in the container. It is then ready for mailing. For sterile collections the rubber nipple, siphon tube and rubber stopper may be boiled.

All of these manipulations are simply and easily carried out, but care should be taken to avoid agitations likely to cause blood cells and serum to mix. The siphon arrangement was made by Harris Comer, of the Steel Glass Company, of Philadelphia, who is in a position to supply complete outfits ready for mailing.

# A CONVENIENT OUTFIT FOR THE SCHICK TEST*

By Augustus Wadsworth, M.D., New York

WHEN the Schick test was first adopted in this country, none of the outfits for its administration were considered sufficiently reliable for accurate or general, state-wide use. Diphtheria toxin for this test is diluted so that 0.1 c.c. of the diluent will contain one-fiftieth M.F.D., or one-fiftieth of the quantity that will kill a guinea pig weighing 250 grams in about four days. One-tenth of a cubic centimeter of this dilution is injected intracutaneously with a fine needle. The toxin is not only very labile and subject to rapid deterioration, but such minute quantities must be so accurately measured that, in sending an outfit to different parts of the state, it was necessary to have one which, besides being convenient, would remain serviceable for some time.

In 1916, Miss Tula Lake Harkey undertook the problem of devising a new outfit before she went overseas as chemist to Base Hospital No. 33. While in England, she developed cerebrospinal meningitis and died, as have so many others, in the service of her country. Work which she did on standardizing an outfit was so successful in leading to practical results that I wish to record her observations together with a description of the outfit, which has now been in use, with very slight modifications, for nearly seven years.

In her investigations, Miss Harkey found that the number of drops in each cubic centimeter of a fluid delivered from different pipettes varied markedly, but when the same pipette was used the variation was not so great; and when a small pipette with a fine point was standardized, the drops were very nearly constant. While she was engaged in her work, Donald[1] and Walker[2] published methods for measuring drops by the use of standard solutions of hydrochloric acid and sodium hydroxide. Following their suggestions, standard solutions of 4N HCl and N/100 NaOH were used as follows: The hydrochloric acid solution was drawn into the pipette, and then one drop of the hydrochloric acid solution falling from the pipette was titrated against the sodium hydroxide solution. Three titrations were necessary for each pipette, and the average of these was taken. With accurate solutions the volume of the drop of acid was found to be one-four-hundredth of the volume of sodium hydroxide used to neutralize the acid. From this volume was calculated the amount of physiologic salt solution required to dilute the toxin so that 0.1 c.c. of the dilution would contain one-fiftieth M.F.D. of the standard toxin.

It was early decided that a second pipette would increase the efficiency of the outfit by providing a reserve in case of possible breakage of the tip of one, and by allowing a second dilution to be made when the volume of sterile

*From the Division of Laboratories and Research, New York State Department of Health, Albany, N. Y.

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salt solution was replaced in the graduated bottle. Later, the advantages of preparing some outfits with more bottles of salt solution became apparent.

Any outfit designed to measure so small a quantity of material with accuracy necessarily requires more careful technic than the average physician is ordinarily obliged to employ. This explains why, at first, some have thought it too complicated. Practice and a trial of other outfits soon change this opinion, especially as the results obtained are most gratifying. From the standpoints of maintaining potency and enabling the laboratory to meet the unequal demands of state-wide distribution, it has proved most satisfactory. The methods employed in its preparation and directions for its use follow.

*The Outfit.*—The outfit consists of a small vial of undiluted diphtheria toxin, a pair of standardized capillary glass pipettes for measuring the toxin, one, three or eight small bottles containing the exact amount of salt solution necessary to give the required dilution when one drop of toxin from the accompanying pipette is added, and a small sterile tube for use in preparing the control test. The outfit containing one bottle of salt solution is designed for general use; those containing three or eight bottles are suitable when many persons are tested at one time.

*Preparation of Capillary Pipettes.*—Five millimeter glass tubing with a rather thick wall is broken into four-inch lengths with ends slightly annealed, thoroughly cleaned and dried, and drawn out to form two capillary pipettes with very small bore. Each pipette is standardized by titrating the drop of 4N HCl it delivers with approximately N/100 NaOH, standardized against the acid.

*Standardization of Hydrochloric Acid and Sodium Hydroxide Solutions.*—The hydrochloric acid and sodium hydroxide solutions are prepared so that the HCl is 4N and the NaOH approximately N/100. The sodium hydroxide solution is then standardized against the hydrochloric acid solution, 1 per cent aqueous solution of alizarine sodium sulphonate being used as indicator. To ensure accurate results two N/10 HCl dilutions are prepared from the 4N HCl and three 2 c.c. samples of each dilution are titrated with NaOH. The titrations must agree within 0.1 c.c. In titrating, the volume is made up to about 70 c.c. with water, and one drop of indicator is added. The end-point is determined by comparison with a control flask containing water and indicator only. This flask is prepared at approximately the same time as the titration flask, as there is some change in color on standing. The value of 1 c.c. of 4N HCl in terms of NaOH is determined as in the following example:

| | | | |
|---|---|---|---|
| Average reading | 2 c.c. N/10 HCl | = | 19.962 c.c. NaOH |
| | 1 c.c. N/10 HCl | = | 9.981 c.c. NaOH |
| | 1 c.c. N/10 HCl | = | 40 c.c. 4N HCl |
| | 1 c.c. 4N HCl | = | 399.25 c.c. NaOH |

*Standardization of Capillary Pipettes.*—The volume of one drop of 4N HCl delivered by the pipette is determined by titration with the standardized NaOH solution. If the variation in the burette readings is not more than 0.05 c.c., three titrations are made and the average is taken, if 0.1, five titrations, and if greater, the work is repeated. The pipette is filled half-full, held

in a vertical position, gentle pressure is exerted upon the upper end until the drop starts to form, when the pressure is removed and the drop is allowed to fall unaided. The first one or two drops are discarded.

Each standardized pipette is cleaned, labeled with the number given it on the record card, and placed in a plugged tube with a cotton guard at the bottom. After sterilization in hot air the pipettes are paired. The volumes of salt solution required must be the same, within a 0.05 c.c. variation, for both pipettes.

The volume of 0.85 per cent salt solution used for diluting a drop of toxin delivered by the pipette so that a final dilution of one-fiftieth M.F.D. in 0.1 c.c. is obtained, is computed as follows:

Assume that the drop of 4N HCl delivered by the pipette is neutralized by 2.9 c.c. of the NaOH solution standardized above, and that the M.F.D. of the toxin to be used is 0.003 c.c.

$$\text{Vol. drop: } 1 \text{ c.c.} = \text{T (titration of drop with NaOH)} : 399.25$$

$$\text{Vol. drop} = \frac{1}{399.25} \times \text{T}$$

$$\frac{1}{10} \times \frac{1}{\text{Vol. salt}} \times \text{Vol. drop} = \frac{1}{50} \times 0.003$$

$$\text{Vol. salt} = \frac{5 \times \text{Vol. drop}}{0.003} = \frac{5}{0.003} \times \frac{1}{399.25} \times \text{T}$$

$$= 4.174\text{T}$$

$$= 4.174 \times 2.9$$

$$= 12.104 \text{ c.c.}$$

The volume of salt solution required to dilute the drop from this pipette is then 12.1 c.c.
General formula:

$$\text{Vol. of salt solution required} = \frac{5}{\text{M.F.D.} \times \text{S}} \times \text{T in which}$$

S = value of 1 c.c. of 4N HCl in terms of NaOH
T = value of drop of 4N HCl in terms of NaOH

The bore of the pipettes selected should be such that the drop falls easily and unaided except for the initial pressure to start its formation. A drop of the proper size, when toxin with an M.F.D. of 0.003 is used, requires from 11 c.c. to 14 c.c. of salt solution.

*Diphtheria Toxin.*—A well stabilized toxin with an M.F.D. of approximately 0.003 to 0.004 is selected. Before being bottled, it is restandardized by the subcutaneous test. Guinea pigs weighing between 250 and 280 gm. are used, at least 75 per cent of which should die with typical lesions in somewhat less than ninety-six hours. If these tests are satisfactory, two guinea pigs are injected intracutaneously with $\frac{1}{50}$, $\frac{1}{250}$ and $\frac{1}{500}$ M.F.D. The first dilution should induce a very marked reaction, the last a reaction corresponding to a definite Schick reaction in a person. Tests upon laboratory workers of known susceptibility or immunity are valuable as controls.

*Bottling of Toxin.*—The toxin is bottled in approximately 0.75 c.c. amounts in 1 c.c. amber vials which tests have been shown to be practically neutral. During the process the toxin is protected from light and warmth as much as possible.

*Assembling of Outfits, Including Bottling of Salt Solution.*—Before the salt solution is dispensed, the pipettes, and labels for the bottles giving the numbers of the pipettes and the volume of salt solution required, are placed in wooden boxes and each outfit is given a serial number. The required volumes of sterile 0.85 per cent salt solution are filled into small bottles, each of which is closed with a rubber stopper and placed immediately in the corresponding outfit. The filled material is tested for sterility. The bottles are capped with fish skin, examined for defects, and marked with a diamond pencil to indicate the level of the salt solution in order that possible leakage may be detected readily, or that the bottle, properly rinsed and sterilized, may be refilled and used again with the second pipette. The outfits are then completed except for the toxin, which is added immediately before they are placed in the cold room.

*Time Limit.*—When an outfit is to be sent out, the "Return date" is entered on the label of the box. In the case of a toxin standardized for the first time for use in the Schick test, this date is three months later than that of the standardization test. About a month before the date of expiration, the toxin in the small bottles is retested. If no deterioration is shown, the time limit on the outfit is extended two months. This procedure is repeated at bimonthly intervals.

In each outfit a circular is enclosed, describing the material and equipment and giving directions for use, from which the following essential points are taken:

*Precautions to be Observed.*—Do not use the outfit without first reading carefully the directions, which should be followed exactly. Keep the outfit in an ice box—the toxin deteriorates rapidly at room temperature or in the light. Always make sure that the number of the pipette given on the bottle of salt solution and the number on the capillary pipette correspond. Do not use a pipette with a broken or nicked tip as any change in the tip will alter the size of the drop. See that the level of the salt solution corresponds to the line on the bottle. Do no use any part of one outfit with another outfit. Never use the diluted toxin when it is more than twelve hours old.

*Preparation of Dilution.*—Under sterile precautions remove the capillary pipette from its container and by gentle suction draw up from the bottle of undiluted toxin enough toxin to half-fill the pipette. Hold the pipette vertically, with the thumb and second finger, and press gently on the top of the pipette with the first finger until a drop begins to form. At once remove the finger and allow the drop—which should be discarded—to fall unaided. Repeat the procedure and allow the second or the third drop obtained in the same way to fall into the bottle which contains the exact quantity of salt solution necessary to make the correct final dilution. Never add more than one drop to the diluent. Cork the bottle and mix the contents thoroughly. Each 0.1 c.c. of the dilution contains the required dose of toxin for the test.

*Preparation of Control Dilution.*—Transfer part of the diluted toxin to the small sterile test tube by means of a sterile pipette—allowing no toxin to touch the upper part of the tube—or by pouring and then flaming the top of the tube thoroughly to destroy adherent drops of toxin. Plug and immerse in boiling water for three minutes.

SUMMARY

A convenient outfit for the administration of the Schick test is described and a detailed account is given of a method of preparing it which has been in use for several years.

Outfits made and assembled as described have been distributed in large numbers during a period of nearly seven years and have been found highly serviceable and reliable.

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## ELECTRIC HEATED FUNNEL FOR FILTERING MEDIA*

By Emil Weiss, M.D., Chicago, Ill.

THE hot water funnels in use for filtering agar and gelatin are slow, wasteful and hard to keep clean. Filtering in the autoclave requires very much time, and excessive heat is usually applied to the media during this period of time. We have made a funnel similar to the hot water funnel, but used electricity as our source of heat. Fig. 1 shows the funnel complete

Fig. 1.

as we are now using it. Fig. 2 is a cross-section of this funnel. The wires (w) are placed on the bottom. The inside of the air space (x) is enclosed with asbestos (A) to prevent loss of heat and also to keep the outside aluminum covering from getting too hot. The funnel is made of aluminum. A glass funnel can be put inside of this if desired. The temperature inside

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the funnel is 90° C. Fig. 3 shows the arrangement of the heating wire at the bottom of air space.

We have been using such funnels for twelve months in the medical school and hospital laboratories. The temperature is constant; there is

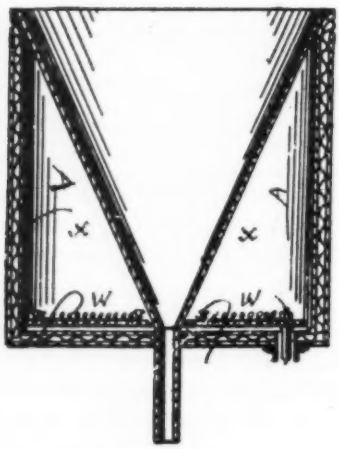


Fig. 2.

Fig. 3.

practically no waste of agar or gelatin; the working space can be kept clean as there is no boiling over of water; there is no danger of fire from exposed gas flames; the filtration process can be quickly started and rapidly performed with such an apparatus.

# *TRANSACTIONS*

## THIRD ANNUAL CONVENTION OF THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

*June 5-7, 1924*

*(Continued)*

### Symposium on Laboratory Procedures

*President MacCarty.*—My object in having this little symposium is this: I do not think that all of the clinicians really appreciate what the laboratory does for medicine. They have had an idea that the laboratory was a thing apart from medicine; that medicine is merely the art of getting the patient, making him feel good and holding him—pleasing the family, in other words. Nevertheless the laboratory still has in the practice of medicine a close relationship. There is nothing vicious in the attitude of some clinicians, but they do not understand what the laboratory is trying to do and actually does accomplish for medicine. The laboratory man on the other hand has not always appreciated the physician's activities. Now, we have come into medicine since the advent of laboratory medicine, and we have spent most of our lives in laboratories, and have not come into close contact with the actual practice of medicine. In other words, we are not in the actual practice of medicine. Laboratory sciences, as you know, are quite recent, and have required that the laboratory man spend considerable time working in the laboratory; thus he has not had the opportunity to take care of patients. I think it is about time, since we have standardized so many laboratory procedures, for the physician to be more intimately familiar with the laboratory procedures and their value, and for the laboratory man to be more familiar with the art of the practice of medicine. It was with this goal in my mind that I had this symposium arranged. We have in this institution, fortunately, many men, young men all of them, who are actively in contact with patients, and who at the same time, being young, have had extensive laboratory experience; we have tried to make laboratory practice, and clinical practice one thing, and not keep them separated. The men who are on this program today are primarily interested in patients, and are also well trained in laboratory procedure. I shall call on Dr. Sanford first.

*Doctor Sanford.*—My few remarks are going to be very impromptu, because I have been thinking the last few days whether you are going to get something to eat, and a place to sleep, and prosaic things of that sort.

I think I will have to put a little "reverse English" on my first shot around the table, and say that I believe we do too much laboratory work now. I think the pendulum has swung a little too far. Twenty years ago we were stressing physical diagnosis and the observation of signs and symptoms. The young man coming to us now wants every laboratory test that he has ever heard of before he is going to hazard a guess as to what is wrong with the patient, or take a look at him, and sometimes it leads him into a rather bad pitfall. I want to emphasize the points that MacCarty has just mentioned. I believe that the laboratory man should also be a clinician and should wherever possible see the patient, acting somewhat as a consultant as to the advisability of doing certain tests.

Laboratory procedures are stressed to a great extent at the Mayo Clinic. In making out the history, the first man that sees the patient writes the history; he has a little yellow slip attached to the history, upon which he puts those things he wants done to aid him in mak-

ing his diagnosis. On the slip is a list of various clinical laboratory procedures. In the few minutes at my disposal I will go down the list of things done in my section and try to make a suggestion, where I can, as to whether they are of very much clinical value. There are certain things we do routinely, and I think that probably their clinical value has become established.

Of course, we do a urinalysis, just the ordinary routine urinalysis. Patients are sent for hemoglobin, and red and sometimes white counts. Gastric analysis: I do not know really how much clinical value gastric analysis has, except to find out whether there is retention. Of course, that statement is a little strong. Then, in bacteriology I would say that clinical bacteriology, the majority of it, is rather negative. Negative results are of value, of course, in throat cases, and so on, and yet a negative finding is negative and that is all we can say about it. Our positive findings in bacteriology certainly are of great aid in diagnosis. Some of the special things we do in blood work,—the fragility test, for instance; if you have an increase in fragility, you have made a diagnosis, when coupled with the physical findings. The coagulation factors: an increase in coagulation time is of great service, usually, to the surgeon. Whether he wants to operate before something is done to alter coagulation factors is often a question to be decided by the laboratory. Plate counts, prothrombin determinations may help in making a diagnosis of hemophilia. Blood grouping: we do a great deal of blood grouping as you know, here. We could not do our transfusions without it by the methods that we use. The surgeon would be lost if we did not have on hand all the time Group IV donors ready for emergency transfusions.

As far as a Wassermann is concerned, Stokes says it is worth about fifty per cent in diagnosis, especially in the field of dermatology. I do not care to start to argue that, one way or the other. We do not do a routine Wassermann here. Much of our attitude toward the Wassermann has been influenced by the dermatologist's attitude toward it. We rather arbitrarily do Wassermanns on patients between fifteen and thirty years of age, not that age has much to do with it, except that Stokes says he can do something for the syphilitic patient between fifteen and thirty years of age, and he is not so sure about those later in life. All patients undergoing operations should have Wassermanns; the surgeons require it of their patients. Of course, we are assuming that the Wassermanns really help them in the diagnosis of syphilis, and according to Stokes it helps him just about fifty per cent. The other fifty per cent he bases on history and his own findings and physical examination. Blood culture: of course, there is nothing much to say about that. When it is positive, it makes a diagnosis. We do considerable blood chemistry; much that is negative. I think this is the age, or perhaps a year or two ago was the age, when blood chemistry was the important thing, and out of it all we have sifted some rather significant clinical procedures. The blood urea now is just about as common a clinical laboratory test as the functional test—but not quite. The functional test comes first, but it is usually now checked with blood urea. It does tell us some astonishing things at times. I used to think it was useless to do a blood urea on patients who would walk into the laboratory, but they do walk in with considérable percentages of blood urea. When the patient is in the hospital, blood urea determination often helps in the diagnosis, not as to uremia, but as to kidney function. Blood sugar: Wilder will stress that a good deal more in his talk. Examination of spinal fluids: there is very little indication for withdrawing the spinal fluid at all except for the relief of the pressure, but the laboratory procedures are often of extreme value in making a diagnosis. The stool examination: we perhaps do more stool examinations than are done in most places. This really is because for the last thirteen or fourteen years we have been very much interested in the fact that our Northern patients had amebiasis or other intestinal parasites, and while they were not of as much importance as in the South, they were of interest. About twenty per cent of our stool examinations are positive for some species of intestinal parasite. Of course, a large percentage of these probably have not very much to do with the patient's symptoms. Often we do find ova of parasites when the patient does not know that he is harboring any of the worms.

Sputum examinations: We do these at the request of the chest specialists. The skin

sensitization test: we are working with the internists on asthmas and hay-fevers, but I do not know that we are up to date, since hearing Black's paper yesterday. I want to take this occasion, if he is here, to congratulate him on the excellent piece of work that he did in that paper. I think we probably are working with a 1923 model, and he has given us the 1925 ideas.

Guinea pig inoculations: we use about one thousand guinea pigs a year. I would say that Braach considers the test of extreme importance in his diagnosis of kidney tuberculosis; and for Henderson, I think it is more in confirmation of diagnosis in tuberculosis of bones and joints, because we get the material after operation.

Sinus examinations: You perhaps know that we have been very much interested in the prevalence of actinomycosis. We have had over two hundred cases here. The examination of old sinuses leads us to the finding of the characteristic "sulphur bodies." Magath has been very successful also in finding of tubercle bacilli in tuberculous sinuses.

The examination of duodenal contents with Schneider's test, demonstrates the increase in urobilin and urobilinogen and helps somewhat in the diagnosis of pernicious anemia, if we accept the theory that the increase in these pigments means the increase in blood destruction. It does go along with it most certainly, and while I would not consider it of great value in diagnosis alone, it is confirmatory.

*President MacCarty.*—The next speaker, Rowntree, needs no introducton, I am sure. I was going to use him as an example of what I think the real physician should be. The first time I met Rowntree was in a pharmaceutical laboratory. I was visiting Baltimore, and he was there. I do not know that he had ever seen a patient or ever expected to see a patient. The next time I saw him he was running around the wards in Johns Hopkins Hospital, and was interested in patients. Here, where I live with him, he does both; he sees a number of patients a day, and is in the laboratory, and he can give a very practical picture of medical laboratory problems.

Rowntree's remarks have been published in the JOURNAL OF LABORATORY AND CLINICAL MEDICINE in the issue of October, 1924, page 75.

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DRS. KOLMER and STEINFIELD read a paper entitled **A Study of the Specificity of the Kolmer Complement-Fixation Test for Syphilis**, published in the JOURNAL OF LABORATORY AND CLINICAL MEDICINE, x, No. 1.

DISCUSSION

*Doctor Sanford.*—Kolmer, as usual, has gone thoroughly into the subject. The only experience we have had statistically is in a paper that he has mentioned. In the five hundred diabetics, we might say that the Noguchi antigen did not give any more false positives than his antigen; that is, both of them gave no false positives. Incidentally, we might say that the incidence of syphilis, judging from the Wassermann reaction, and the history, was really lower in these diabetics, over five hundred of them, than is the incidence in the average run of patients that registers at the Mayo Clinic. I am not trying to argue against the statements of some men that syphilis has something to do with diabetes, but our findings do not justify such an opinion. For years we have had all our serology on patients that have been fasting, and for many reasons, we will continue to do that in the future. We bleed our patients for blood chemistry at the same time. They come at eight o'clock without their breakfast, and they get through in time so that they can get their breakfast. That gives us very clear serum, if nothing else. I have no data to prove the point as to the statement of some serologists, and a few experiences of mine, that false positives might have occurred from the blood being loaded with the recently absorbed products of digestion. With our own antigen, we also felt occasionally that we did get false positives in miliary TB., or in carcinoma cases. We have not noticed this, however, at all in the short time we have worked with the Kolmer antigen.

There is one other thing that I would like to put into this discussion, if I might, although it is not quite germane to the subject, and that is the anticomplementary serum, not

due to contamination from bacteria, or due to age, but the fresh serum that is anticomplementary. We have tried titrating out the control tubes, that is, putting in the same varying quantities, as we do with Kolmer's quantities, in the tubes without antigen, and we still find that this anticomplementary substance will fix complement in a high degree of dilution. This occurs in patients that we know are syphilitic antibody, and we have felt when we got a serum of this kind it meant syphilis. Of course, it is not "according to Hoyle." Our controls did not clear up, and I would like to know some of your opinions about anticomplementary serum.

*Doctor Ives.*—I would like to discuss the paper briefly. I believe that we may infer that Kolmer believes that there is an inherent defect in the ordinary cholesterinized antigen. He believes that the ordinary antigen gives nonspecific positive reactions in certain conditions. Such reactions may be interpreted in another way. To me the all-important point is not the kind of antigen used; of equal importance is the proper use of the antigen and of other reagents of the test. I have no reason to believe from my work that cholesterinized human heart antigen gives false positives excepting in very rare instances.

In regard to the matter of anticomplementary sera: Sera which are sufficiently anticomplementary to prevent a report are most rare. However, with positive sera there is often an inhibition in the rate of hemolysis when compared with negative sera.

*Doctor Ruediger.*—Now, with regard to the positive Wassermanns in the various diseases, I have had the opportunity of testing 150 lepers with a uniformly negative result in all excepting the syphilitic lepers. At the same time, there was a medical officer from the navy, running a parallel series of 150, and he got negative results in all but the syphilitic lepers. About the same time a medical officer of the army was running another series of lepers, and he got 75 per cent positives. The army officer's paper went as far as the Board of Censors, and disappeared. The reason for these positive results was very clear. He used an alcoholic extract of dog heart muscle, and those of you who have tried dog heart muscle, or guinea pig heart muscle, probably got many false positives; at least, I did, as well as others that I have seen working with it. The heart muscle of a dog, or the heart muscle of a guinea pig is not suitable for the preparation of an antigen. I had many cases of frambesia, and they all gave as strongly positive results, as the syphilis does. I tried all the diseases that I could obtain and the only one I did not get was human trypanosomiasis. I have not tested out any of that. Now, as regards the question of anticomplementary serum; I have not yet found an uncomplementary serum that I could not test. Sometimes the anticomplementary property completely disappeared on heating, and if that did not do it, I reheated it at intervals of two or three days, and by the third heating usually it completely disappeared; or one can do it easily by simply running a control tube for every antigen tube, using the human serum in decreasing quantities, because in such cases the specific fixation will always go much further than the anticomplementary property can be detected.

*Major Falisi.*—I am afraid that the previous speaker (Dr. Ruediger) has left an incorrect impression concerning the Wassermanns as performed in the army.

The official technic is completely described in Colonel Craig's work, with which most of you are familiar, and needs no apology.

Another point upon which I wish to touch is the question of chyle in serum. During my experience at a recruiting depot where things were moving rapidly, it was necessary to take bloods about one and one-half hours after the noon meal and many of these bloods showed chyle, which we feared would interfere with the reaction.

As a matter of fact, the anticomplementary and positive reactions were no more numerous than with the clear serums. Every positive reaction was investigated and only rarely was it impossible to elicit a history

*Doctor Kolmer* (Closing.)—In reference to Sanford's discussion on the influence of digestion upon serum collected soon after eating, it is my impression that these sera may be at times slightly uncomplementary, but I agree with the last speaker that in Philadelphia we pay little or no attention to this condition, and that the results are uniformly

satisfactory, even with sera which are markedly chylous in appearance. I doubt, however, whether we correctly understood Sanford's reference to anticomplementary sera. I happen to have the advantage of understanding completely, because he was good enough to correspond with me about the matter. Sanford has in the last year or two met with sera from one or two individuals where even fresh sera were anticomplementary. We have met with the same condition twice in the last few days. I believe that it might be related to certain tissue changes that are produced by the administration of the arsphenamines, but it is a subject that requires very careful investigation. Our experience has been similar to that of Sanford, in that these sera have come from cases under treatment. I can assure Ives that no one entertains a higher opinion of cholesterinized antigen than myself. On the other hand, I believe that the ordinary saturated extract of heart muscle that contains approximately four-tenths of one per cent of cholesterol is capable of yielding more nonspecific reactions than the plain alcohol extract. It depends, just as Ives has correctly stated, upon the technical conditions. If the serologist will refuse to accept any cholesterinized extract in which the dose is more than one-fourth or one-fifth of the anticomplementary dose, in other words, if the dose employed is not more than one-tenth of the uncomplementary unit, I agree with him thoroughly that the danger of nonspecific reaction is almost negligible; but, on the other hand, if one employs an extract of this kind in which the dose is about one-half or one-third of the anticomplementary unit, it is my conviction that it increases the danger of nonspecific reactions. In my new antigen I employ cholesterol; but if we refer to the original work of Sachs, we will find that he advocated not more than one-tenth or two-tenths per cent and not the four-tenths that Swift advocated in this country. It has been our experience if we add one or two-tenths per cent cholesterol to an alcoholic extract, that we get all the virtues of cholesterol without increasing the risk of increasing its nonspecific reactions; so we use cholesterol in this amount in my new antigen. There can be no warmer advocate of this practice than myself, but only in two-tenths of one per cent rather than four-tenths. I thoroughly agree with Ruediger about alcohol extracts of guinea pig heart. Our experience has been similar to his own in that these extracts are frequently highly anticomplementary and inferior to extracts of human or beef heart muscle.

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Dr. H. E. Robertson, Rochester, Minn., read a paper entitled **Better Postmortem Service.** (For original article see page 486.)

### DISCUSSION

*Doctor Kolmer.*—I, of course, with all here present, feel that every point brought up in this remarkable paper is worthy of discussion; but there is one point in particular that I think is worthy of more than usual attention, and that is the attitude of the undertaker. In Philadelphia, we lose, in the hospitals of the Graduate School of Medicine, a large number of autopsies by reason of the fact that the undertaker gets in touch with the people first, and convinces them that if the doctors perform an autopsy it is not possible for him to guarantee the proper preservation of the body. With the better class of undertakers, we have no trouble at all, because with the care mentioned by Robertson it is easily possible for us to leave the body in such a shape that a good undertaker can preserve it perfectly for burial. The idea, however, occurred to me during the past year that it may be well for us to investigate the kinds of embalming fluids that were being employed to see if we could employ an embalming fluid which the undertaker could first use for the embalming of the body, to be followed by the autopsy. Now then, an embalming fluid of this kind must not produce any gross changes in the organs, nor should it produce histologic changes. In an investigation of the embalming fluids being employed in Philadelphia, we found that all of them were so loaded with formaldehyde that it was impossible to use them for embalming, preliminary to the necropsy, by reason of the profound changes produced in the color and consistency of the organs; but I would like to ask Robertson whether he knows of any fluid that can be used by the undertaker before the body is necropsied? In Philadelphia,

I have been using a fluid that was devised by Hewson, our professor of anatomy in the Graduate School of Medicine, which he employs for the preservation of bodies for anatomic purposes. This fluid preserves the body indefinitely. It contains only one and one-half per cent of formaldehyde, and I now have my autopsy room equipped with all the apparatus which the undertaker requires, and also with this fluid, and whenever I have objections raised by an undertaker, or with the family with whom I am dealing, I simply tell the undertaker to please come in and I will give him all the facilities that he can possibly wish for, including the preserving fluid, and that he is to proceed with the embalming of the body just as if no necropsy were to be done. The only objection to this fluid that has developed in practical experience is the fact that the percentage of formalin is so low that it does not fix the muscles of the face, and after a day or two the jaw begins to drop, so I ask the undertakers to use their own fluid for injecting the head. Dogs embalmed with this fluid have been kept in the basement of the laboratories in close proximity to steam pipes for a matter of three months until they became mummified with absolutely no odor or decomposition. Our experiments are still under way, but we hope that others will take up this matter in a spirit of friendly cooperation with undertakers for the purpose of increasing our percentage of necropsies.

*Doctor Robertson* (closing).—Kolmer mentioned the undertakers more in detail. I am glad he did. We started a school for embalmers at the University of Minnesota. We had a conference in which the undertakers and the doctors presented their sides. I used to take the undertakers down to the morgue and perform postmortems, and then ask an undertaker who I knew was qualified to repair the body to show them how it should be done. Any such conference could be held at any place, and it is of tremendous value. Sometimes these poor fellows do not know how to repair the bodies after examinations have been made. In regard to his point about the fluid, this is liable to happen more with the inexperienced embalmer. He could make a good fluid himself just as well as he could buy it, but he is a good deal like other people who like to buy their "Quaker Oats" in packages, so he buys his stuff in expensive bottles. Now, one of the simplest means of avoiding this trouble is to tell him if he will use the solution, that is one-half the usual strength, he will find that he will wash out the blood more easily, that he will get a more intensive penetration of the capillary tree than he would with his stronger solution, and that he will be more pleased with the result; and then tell him that if for some reason or other the cheeks are not fixed tightly enough, if he injects the usual strength of his embalming fluid in the arteries, the fixation will be good. Embalming does not depend so much on the strength of the fluid as it does on the wide distribution in the capillary tree.

Brown brought up an illustration of the exact point I wish to make, and I do not want to be offensive, because we are dealing with extremely difficult things to talk about frankly, but we have got to be frank with a thing like this. Why is it that this medical student did not want to have a postmortem examination on his father, and why is it that Brown agrees with the medical student about that? Because an autopsy is not conducted under a decent sort of régime; because it is not done with due respect to the dead. If it is not done decently and in such a way that a relative could come in and see it, why of course, it is objectionable. Relatives occasionally say to us, "I will give you permission to do an examination if you will let me come in and see you do it". I say, "Sure, come along, we will be glad to have you, and we carry out the usual examination. If it is done decently, there can be no objection; and no postmortem ought to be done unless it is done decently. In other words, I do not like to have fellows enter the room with their hats on; I disapprove of smoking in the room; of blood dropped on the floor; of disagreeable sights more than are necessary, because I want to treat that individual just as I want my own body to be treated when an examination is made upon me, and such an examination is going to be made if I can persuade any doctor to do it. Now, it is that attitude towards the examination that will properly educate the physician and the medical student, and will change the thought of such a type as Brown mentioned. It will change this aversion toward a postmortem examination.

*Doctor Brown.*—An interesting resolution relative to postmortem technic was pre-

sented to the Rochester Medical Association at Rochester, New York, by the local undertakers' association recommending that while doing an autopsy the large vessels be ligated low enough to permit the undertaker to embalm the body satisfactorily. Acting on this recommendation all pathologists in Rochester are carefully tying off all the large vessels before severing from the aorta.

There is one point which was brought up by Robertson on the sentimental side of relatives of deceased persons that has another side as I see it. A medical student who goes through his four years in a recognized medical school is able to visualize the entire picture of a postmortem examination, and it so happens in his case that in the event of the death of one of his relatives he is able to picture to himself the complete postmortem examination in all details as the request is made for permission. If the examination is made he is really subjected to double grief and it seems to me it is rather hard on some types of trained medical men to ask them to suffer twofold unless the case warrants it. Now, the layman does not have to go through quite the same experience; he has in his mind no picture of what is to take place and he suffers from the death of the relative alone.

A friend of mine told me of an autopsy carried out in an undertaking room several years ago that shows how a postmortem examination may react on a relative even after he has given his consent to the same. The day was warm and the door of the examining room was left open though unfortunately it was not guarded. The relative paced back and forth on the walk and finally walked around to the back of the building and casually looking in through the door, saw the operator at work on the autopsy that to him was most gruesome. The shriek that came from his lips was that of extreme agony and pain and one that none of us would care to hear. Here of course, the whole plan was wrong but it brings to our attention the need of the greatest care in the conduct of our postmortem studies.

I think we have got to be careful and tactful, considering the general conditions and obtain permission on first, the cases dying of causes unknown; second, on the doubtful or partly obscure cases and lastly on the routine cases with clean cut diagnosis of the causes of death.

It seems to me that tact is most worth while in obtaining postmortem examinations and the impression should be definitely left with the relatives of the deceased whenever it is possible to do so that a favor is being conferred upon them by making the examination and that it is not being made to satisfy the curiosity of a few physicians and students. The body in the postmortem room should be treated with the greatest consideration; first as a mark of respect to the dead who was beloved by some one; second, that the embalmer may have a chance to restore the appearance so that no observer can see the lines of incision.

The great purpose of medicine is to prevent and relieve human suffering and it seems to me that the suggested wholesale postmortem examinations would be contrary to the fulfillment of this aim and many needless routine autopsies would be made under this policy that would on completion have added not one item of interest, knowledge or satisfaction to anyone except perhaps the statistician.

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## EDITORIALS

### *Some of the Recent Work on Insulin*

AMONG the problems of metabolism, which it has been possible to attack from somewhat new aspects by the use of insulin, are two by the investigation of which certain results of considerable interest have come to light. One of these problems concerns the physiologic condition of depancreatized animals kept alive by daily injections of insulin, and the other the possible derivation of this hormone from other organs than the isles of Langerhans.

In a paper published a year ago in the British Journal of Experimental Pathology, by Allan, Bowie, Macleod and Robinson, it was pointed out that although depancreatized dogs could be maintained in excellent nutritive condition for periods varying from two to four months by the daily administration of insulin, they then succumbed to symptoms of acute hepatic breakdown, namely jaundice, bile in the urine and blood serum, entire loss of appetite, vomiting, increase of temperature and rapidly developing weakness.

These symptoms could not be ameliorated by clearing out the bowels, and after the death of the animal they were found to be associated with an intense fatty infiltration of the liver, unaccompanied by serious lesions in the kidneys—except in one of the four cases—or by any significant histologic change in the voluntary or cardiac musculature. It was concluded that the pancreas must be essential to the physiologic well-being of the animal, quite apart from its rôle in producing insulin. Two reasons were suggested to explain how the absence of the pancreas might cause the fatty changes in the liver; the one being that owing to the absence of pancreatic juice a faulty digestive process would be set up in the intestine, and as a result toxic amines, or similar substances, by their absorption into the portal blood, would injure the liver cells and so lead to fatty changes; and the other, that some pancreatic hormone (other than insulin) necessary for the metabolism of fat had been withdrawn from the organism. In support of the former view it was observed that about fifty per cent of ingested protein reappeared in the feces, often in a completely undigested state and that only a small percentage of fat was absorbed. To this extent the observations lent support to those of Lombroso who, in 1910, concluded that the presence of the pancreas in the body is essential for proper absorption from the intestine, even when it does not actually secrete any digestive juices—a conclusion, however, which had been denied by McClure, Vincent and Pratt.

To put these suggestions as to the cause of the symptoms to the test of experiment the next step was to administer to depancreatized animals not only insulin but the digestive enzymes of the pancreas as well. This has been done by feeding raw pancreas to two dogs, with the significant result that one of them has now survived the pancreatectomy for over fifteen months and the other, for over eight months, both being at the present time in excellent nutritive condition. The daily diet in both cases has consisted of from 200 to 400 grams of meat and fifty grams of raw beef pancreas with from 50 to 100 grams of cane sugar, the dose of insulin, given twice daily, being varied in each case between fifteen and twenty clinical units. There is no doubt that by giving the raw pancreas, the absorption of both protein and fat has been very greatly improved and the absence of any symptoms of hepatic insufficiency may possibly be explained by the fact that no toxic products, due to a faulty (microbal) type of protein digestion, are being absorbed into the blood of the portal circulation. On the other hand, this result does not negative the second suggestion, for it may be that by feeding raw pancreas some hormone, other than insulin, which is not destroyed during the digestive process, is absorbed from the intestine into the blood. To test this possibility, we are at present giving depancreatized dogs trypsin in place of raw pancreas, but the results are not sufficiently forward to report at the present time.

We have attempted to investigate the problem in another way, namely by measuring the amount of esterase in the blood. By use of the stalagmometer method of Rona, we have found in depancreatized animals, that instead of being decreased, this blood-enzyme is decidely increased in amount. But

it must be remembered that there is probably both an esterase and a lipase in blood and before we consider this part of our work as complete, the blood lipase instead of the esterase must be measured.

Several other facts of possible clinical interest have come to light as a result of these observations. For example, it has been found that the fasting blood sugar is usually about 0.300 per cent, indicating that the animals are more or less constantly hyperglycemic and yet, apparently, without any untoward effects. This does not imply that it would be safe to allow a human diabetic to carry so high a blood sugar, for there is an essential difference in the clinical condition as it exists in him and in the depancreatized dog; namely, that the diabetic patient still retains some islet tissue which must be protected from overstrain by keeping the blood sugar as low as possible. What the observation does show is that increased blood sugar per se does not cause the degenerative changes which some clinicians have imagined it to do.

Another fact of interest is that one of the dogs (9 months) has gone through a normal pregnancy and is now bringing up two of her family of five. She went through the pregnancy without any perceptible change in the sugar balance but a few hours after delivery she developed severe symptoms of hypoglycemia from which, however, she spontaneously recovered. It would appear that the drawing of sugar from the body through the milk was responsible for these symptoms. In view of the belief, due originally to experiments by Carlson and Drennan, that the developing fetuses may secrete sufficient insulin to protect the mother against the effects of pancreatectomy, the foregoing observation is of great interest. It does not, however, entirely disprove this hypothesis for, although in our experiments no perceptible change occurred in the carbohydrate balance during the pregnancy, it may be that this was because so much insulin was being given in any case that the small contribution of the fetuses could not make itself felt. Since the glucose equivalent of insulin is so very much greater with small as compared with large doses (Allan), the fetal contribution of this hormone, in the absence of injections of the hormone, might have a pronounced beneficial effect on the mother.

Finally, it should be pointed out that there is every indication that these two animals are completely depancreatized, for both of them become intensely diabetic whenever the injections of insulin are discontinued. It is very significant also that in three days and over, after withdrawal of insulin and food from other similarly treated animals the blood sugar has been found to be decidedly higher in fat as compared with thin animals. The question arises whether this may indicate the derivation of sugar from fat (Chaikoff, Markowitz and Simpson).

Another problem of clinical interest concerns the source of insulin in the animal body. The isolation of substances capable of lowering the blood sugar of normal animals from all sorts of materials, both animal and vegetable in origin (Collip, Winter and Smith, Best, etc.), and even from the tissues of diabetic animals (Best and Scott), naturally raises the question as to whether the isles of Langerhans of the pancreas can, after all, be the

exclusive source from which the intact animal derives this hormone. On account of these facts some authors, notably Oertel and Swale Vincent have formally challenged the islet hypothesis of diabetes. Supplementing the excellent anatomic and pathologic evidence upon which originally this hypothesis was built, three proofs of a different character arising out of the present work may be added. The first of these is the fact, alluded to above, that the discontinuance of the injection of insulin into depancreatized dogs causes severe symptoms of diabetes to supervene within a day or so, followed by death in a remarkably short period of time unless insulin is again administered. If tissues other than the pancreas can produce insulin why do they fail to do so under these conditions? The insulin that is extractable from them must be incapable of functioning in the depancreatized animal and pancreatic insulin only can prevent diabetes.

The second and third proofs have been obtained by work on certain of the bony fishes (Teleostei) such as the angler fish (Lophius piscatorius) and the sculpin (Myoxocephalus) in which the pancreas does not contain the usual islets of Langerhans, these being collected into encapsulated glands of considerable size, situated outside the pancreas, usually in the neighborhood of the spleen and the pylorus. Two years ago, I found that extracts prepared in the usual way from these principal islets, as they are called, contained very large amounts of insulin whereas none was obtainable by exactly the same technic from the pancreas itself. McCormick and Noble have also prepared large amounts of insulin from the principal islets of the cod and pollock. More recently, Swale Vincent, Dodds and Dickens have found that *some* insulin may be prepared by the picric acid process of Dudley from the pancreas of these fishes but they admit that the yields obtainable are very small compared with those from the islets. It may be that the few scattered microscopic islets, observed by Slater Jackson and by Scott in the pancreas itself are responsible for these effects. But even this overwhelming presence of insulin in the principal islets as compared with the pancreas proper is not absolute proof of the insular hypothesis, and since we may consider the present as a test case, not only of the islet hypothesis, but also of the general principle that difference in function is often related to essential differences in structure, McCormick and I have attacked the problem in another way. In the sculpin it is possible, by performing laparotomy, to excise the principal islets without much trouble. The operation lasts from fifteen to twenty minutes and the fish soon recovers from its immediate effects and swims about in a normal manner when returned to sea water. The blood sugar measured in fish removed on various days following the isletectomy was found to be markedly raised, indicating the existence of severe diabetes but before attributing this to the isletectomy it was necessary to take into account the fact that for some days (four to five) after exposure to air the blood sugar—normally about 0.035 per cent in this fish—is apt to be raised (asphyxial hyperglycemia). We found, however, that it was decidedly higher in fish from which the islets had been removed and more decisive still, that the hyperglycemia continued after the fifth day, by which time that due to

asphyxia had almost invariably disappeared in unoperated fish. There can be no doubt that removal of the principal islets without disturbance of the pancreas causes marked hyperglycemia in the sculpin and since this is the cardinal symptom of diabetes it seems justifiable to conclude that the fish has become diabetic. In support of this it may be mentioned that the liver of the diabetic fish was found to contain distinctly more fat and less glycogen than those of the controls.

The final proof is still lacking, namely to see whether administration of insulin to the isletectomized fish would prevent the development of the diabetic symptoms. This experiment we hope to do at the earliest opportunity. Meanwhile, however, we have investigated the effect of insulin on normal fish (sculpin) or on fish rendered hyperglycemic by asphyxia and in neither case have we been able to show that it has any very marked influence in lowering the blood sugar. This is strange and may be related to the low body temperature which allows the excess of injected insulin to be excreted before it can act or may be due to a mobilization of sugar out of glycogen or other sources which proceeds at the same rate as insulin can act.

—*J. J. R. M.*

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## *Variation of Virulence in Tubercle Bacilli*

IF we lacked evidence that tubercle bacilli vary in virulence we should be sure that our observation was at fault, since it is certain that all living organisms vary in all their attributes.

As a matter of fact the evidence is plentiful. The characteristic differences which the human, bovine, and avian types show in their power to produce disease in several animal species are well known. Again these characteristic differences are known to be variable, inconstant in degree.

The greatest volume of evidence on this point relates to the "human" type or group. The members of this group are characterized in a general way by slow but plentiful dry growth on media containing glycerin, and by relatively low virulence for cattle, rabbits, and birds. Most, but not all of them possess rapidly lethal virulence for the guinea pig, the animal most used in studying them.

As a rule, strains of this type kill guinea pigs in about two to three months after subcutaneous inoculation. Within wide limits the number of bacilli injected seems to have little effect on the duration of life,[1] though very small numbers of bacilli from virulent strains may cause a comparatively chronic disease.[2, 3] But a number of strains have been reported which even in large doses caused death only after many months or not at all.

The Saranac Lake R1 strain, one of the best known human strains of low virulence, was recovered by Trudeau in 1891 from a case of general miliary tuberculosis. At first it showed standard virulence for guinea pigs, but after two or three years its virulence declined greatly, and now for some thirty years all cultures made from it have shown very low pathogenicity. It has

been extensively studied by Trudeau,[4] Krause,[5] and Baldwin and Gardner.[6] In the great majority of instances it produces in guinea pigs a very benign glandular tuberculosis which shows a marked tendency to heal, and does not interfere with the general well-being of the animal or shorten its life. However, this single strain exhibits variation in virulence, for Baldwin and Gardner observed fatal, though chronic, infections after both inhalation and subcutaneous inoculation, and Rosenberger[7] after intraperitoneal. We have observed a fatal case of fifteen months' duration after subcutaneous injection. Inoculation with tissues and cultures from this animal caused a disease more rapidly fatal than the original R1 infection, but in some cases very much less so than the usual experimental infection.

Numerous other more or less attenuated strains have been reported by Jousset,[8] Arloing,[9] Raw,[10] and others. Dreyer[11] has worked with a strain cultivated by Schottelius, which usually killed guinea pigs in four to ten (commonly six to eight) months. We have reported the production of chronic tuberculosis in guinea pigs by implantation of incubated tuberculous tissues.[3] From such a case a strain has been cultivated which exhibits rather low virulence, though again the disease produced is less chronic than that in the original animal. This strain differs in its history from other avirulent strains, most of which have resulted from prolonged cultivation. We have produced chronic guinea pig tuberculosis by inoculation with dry cultures—a well-known possibility—and also by injecting sputum from a patient with an old fibrous pulmonary tuberculosis in which the bacilli are constantly very long and beaded. Studies of the varying virulence of different strains and of different cultures of the same strain are being continued by Boissevain and Ryder in the laboratories of the Colorado Foundation for Research in Tuberculosis.

The whole question of loss of virulence and possible recovery of virulence has assumed vital importance owing to Calmette's recent revival of the subject of protective vaccination with apparently avirulent strains of tubercle bacilli.[12, 13, 14, 15] Calmette reports that after prolonged cultivation on bile-potato-glycerin media the bacilli lose all power to cause disease when inoculated intravenously or by any other route commonly employed, but remain alive in the body for a year or so, and that during this time the body is refractory to virulent infection, provided it has never been infected before. The behavior of the R1 strain leads one to feel that there may be considerable risk in this procedure.

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[13]Calmette, Boquet, and Nègre: Ann. de l'Inst. Pasteur, September, 1921, xxxv, 561.
[14]Calmette: Brit. Jour. Tubere., July, 1921, xv, 103.
[15]Calmette: Commun. à l'Acad. de Méd., Paris corresp., Jour. Am. Med. Assn., August 9, 1924, 453.

*—G. B. W. (C. T. R.)*

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# *BOOK REVIEWS*

(Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.)

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## *Basal Metabolism in Health and Disease**

BY far the most comprehensive and generally useful single volume on this subject which the reviewer has as yet encountered. The work begins with a brief historical sketch followed by a summary of the salient points of the metabolism of carbohydrate, fat and protein. Those laws of physics which have a bearing upon the performance of metabolism tests are briefly recapitulated. Internal and external respiration are described in some detail. There next follows an enumeration of general principles governing the determination of basal metabolic rates with detailed description of the various instruments in clinical use, and a discussion of their relative values. The normal basal metabolism receives detailed consideration, while nearly 200 pages are devoted to metabolic alterations in disease. Of especial importance and interest aside from thyroid disease is the author's description of the metabolism in diabetes mellitus.

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## *General Cytology†*

THE Marine Biological Laboratory at Woods Hole, Mass., is known to all students of biology especially as a place where one may study the relatively simple forms of marine life so that we may learn more of the fundamental biologic laws of nature. Investigators from many parts of the country customarily spend their summers at Woods Hole, where with thoroughly adequate facilities each studies that particular biologic phenomenon which has for him the greatest interest and presents the most alluring problem.

In 1922 a group of these individual investigators initiated collaborative studies with a view to producing a compilation of monographs dealing with

*Basal Metabolism in Health and Disease. By Eugene F. Du Bois, M.D. Medical Director, Russell Sage Institute of Pathology; Associate Professor of Medicine, Cornell University Medical College. Cloth. 79 engravings. Pp. 372. Price $4.75. Lea & Febiger, Philadelphia and New York, 1924.

†General Cytology. For Students of Biology and Medicine. By Robert Chambers, Edwin G. Conklin, Edmund V. Cowdry, Merle H. Jacobs, Ernest E. Just, Margaret R. Lewis, Warren H. Lewis, Frank R. Lillie, Ralph S. Lillit, Clarence E. McClung, Albert P. Mathews, Thomas H. Morgan, Edmund B. Wilson. Edited by Edmund V. Cowdry. Cloth. Pp. 754. Price $7.50. University of Chicago Press, 1924.

the life processes of the living cell. The result is a 750 page volume entitled General Cytology. The individual writers have incorporated their own work into a general critical view of the literature so that each subject has been covered in every detail.

In the volume one may find most of what is known today regarding the living cell, including its physics, its chemistry, both general, colloidal and biochemistry, methods of fertilization, growth and cellular differentiation, with comprehensive discussions of the theories of heredity as applied to the individual cell.

The chief value of this work will be as an authoritative handbook for students of biology, both general and medical, and as a reference volume for all whose interest is in the general biologic sciences.

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## *Basal Metabolism**

A HANDY reference manual written especially for those interested in the clinical aspects of basal metabolic rate determination. The principles of direct and indirect calorimetry are discussed. The physiology of heat production and heat regulation is presented in considerable detail and the effect of certain drugs thereon is briefly summarized. Chapters are devoted to study of the metabolism in hyper- and hypothyroidism and in various other diseases. The appendix contains tables and charts, helpful time-savers in the performance of metabolism tests.

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## *The Examination of Patients†*

A THOROUGHLY valuable handbook for those who desire to perform exhaustive physical examinations. Much additional space might well have been given to the recording of the anamnesis and the various subjective symptoms, for this indeed must be reckoned a part of the examination. Again, we should have liked to have seen more detailed discussions of the various special examinations, such as pelvic examination, proctoscopic examination, examination of the visual fields and other similar procedures which the internist often through a feeling of inexperience refers to some other specialist without having first undertaken himself.

These are the special fields in which the internist feels a need for a sufficiently detailed reference work applied particularly to his own requirements. It has been very well covered in the author's description of the neurologic examination.

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*Basal Metabolism. By John T. King, Jr. Cloth. Pp. 118. Price $2.50. Williams & Wilkins Co., Baltimore, 1924.

†The Examination of Patients. By Nellie B. Foster, M.D., Associate Physician to the New York Hospital; Associate Professor of Medicine at Cornell University, College of Medicine. Pp. 253. Illustrated. Cloth. Price $3.50. W. B. Saunders Co., Philadelphia, 1924.

# The American Society of Clinical Pathologists

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## The Next Annual Meeting Will Be Held in Philadelphia, May 21-23, 1925
## Prepare for the Coming Convention

A letter and questionnaire have been sent out to all our members apprising them of our next annual meeting which is to be held May 21, 22 and 23, 1925, in Philadelphia, the home city of our President, Dr. Kolmer. Under his inspiration the gathering next year bids fair to excel our previous successful conventions in scientific contributions to our specialty and advancement of our cause. A new and useful feature will be the commercial exhibit of instruments, apparatus and reagents for the laboratory worker. Members should make plans now for attending our next meeting. Dr. Burdick will be pleased to make hotel reservations not only for the Philadelphia meeting of the A. S. C. P. but he is also in a position to extend the service to include reservations in Atlantic City for the A. M. A. convention which is held the week following ours. Those who have papers to present, and we hope there will be many, will communicate at once with the secretary, Dr. Ward Burdick, 652 Metropolitan Bldg., Denver, Colorado